In Brief

Fusion and Fission of Mitochondria

Fuzzy Onions and Fusion

Mitochondria exist in many cells as more of a continuous reticulum than the isolated boats-in-the-ocean depicted in textbooks. They get that way through fusion. This is no mean feat, given that mitochondria have two membranes, which adhere but remain physically distinct. (Nuclei also have two membranes, but the membranes are continuous at the nuclear pores.)

Drosophila that lack the Fuzzy onions protein have defective sperm because the sperm mitochondria fail to fuse. Hermann et al. investigate the budding yeast homologue of Fuzzy onions, Fzo1p, and find that its loss causes the yeast mitochondrial reticulum to fragment (page 359). Cells lacking Fzo1p are viable, but their mitochondria cannot fuse after mating.

Fzo1p is an integral membrane protein with its GTPase domain in the cytoplasm. Fractionation suggests that it spans both mitochondrial membranes, perhaps at the contact sites where inner and outer mitochondrial membranes are directly apposed. Heptad repeats in the cytoplasmic domain may mediate self association in docking, fusion, or both, and two hydrophobic peptides are candidate fusion peptides.

Dynamin-related Proteins and, Possibly, Fission

Screens for budding yeast mutants with strange mitochondrial morphologies reveal that mitochondrial anchoring and movement require interactions with the actin and intermediate filament cytoskeletons. Now Otsuga et al. report that a mitochondrial morphology mutant is defective in the *DNM1* gene, which encodes a dynamin-related GTPase (see page 333).

Dynamin is involved in endocytosis; by itself it can form multimeric spirals that pinch off vesicles. But in their *dnm1* mutants, Otsuga et al. see no defects in endocytosis or in the structure of various membrane-bound organelles. What they do see is a rapid collapse of the highly branched mitochondrial reticulum to form long tubes that remain near the cortex. The mitochondria are still active in respiration and protein import and are correctly distributed during mitosis.

Fractionation shows that a small amount of Dnm1p is loosely associated with mitochondria. By immunofluorescence, the protein is both cytosolic and in punctate structures at the cell cortex. These patches overlap substantially with the tips, sides, and branch points of mitochondria.

In COS cells, mitochondria are normally in a perinuclear array (probably by default because this is where the cytoplasm is thickest) with tubular extensions to the cell periphery. Smirnova et al. (page 351) find that a dominant interfering dynamin-related protein (Drp1) causes the mitochondria to collapse into a perinuclear aggregate. Mutant dynamin has no such effect. An earlier study suggested that Drp1 was involved in secretion, but Smirnova et al. see no effect on secretion with their Drp1 mutant.

There are several possible models for Dnm1p and Drp1 action, and although the two proteins are 46% identical, there is no guarantee that they are acting in the same way. The cortical patches of Dnm1p suggest that this protein could anchor mitochondrial branches to the cortex. Either protein could direct branching or movement of mitochondria.

But the dynamin connection suggests an alternative. Perhaps mitochondria need to be broken up into manageable pieces before they can be distributed around the cell. If so, Dnm1p and Drp1 would be excellent candidates for proteins that could form spirals and direct pinching off of mitochondrial fragments.

"We have no evidence that there is less or more mitochondrial fission with mutant Drp1," says Alex van der Bliek, senior author of the Drp1 paper. "But scission by Drp1 is the most logical interpretation based on the knowledge of spiral formation by dynamin."

How the Extracellular Matrix Keeps Cells Alive

In tissue culture and wound repair, a combination of growth factors and contact with extracellular matrix (ECM) keeps cells alive. But cells in tissues see plasma, not serum, and the anchorage-dependent ECM signal may be the most important survival signal. On page 547, Ilić et al. show that, in the absence of serum, an ECM survival signal mediated by focal adhesion kinase (FAK) suppresses an apoptotic program dependent on p53.

In fibroblasts, Ilić et al. show that this apoptosis can be prevented with a dominant-negative carboxy-terminal p53 fragment that contains consensus phosphorylation sites for protein kinase C (PKC). Mutation of the phosphorylation sites prevents the suppression of apoptosis. Ilić et al. also use inhibitors to show that the apoptotic pathway involves activation of cytosolic phospholipase A_2 ($cPLA_2$), which produces arachidonic acid to activate PKC λ/ι. How $cPLA_2$ is first activated (in the absence of ECM or FAK) is not clear. FAK is activated by ligation of integrins at focal adhesions, and somehow it then shuts off the $cPLA_2$–PKC λ/ι–p53 apoptosis program.

Soluble death signals like Apo-2L and the FAS ligand initiate apoptosis by activating the large prodomain caspases. Inhibition of these caspases with CrmA does not prevent apoptosis of cells lacking an ECM survival signal; the two apoptosis pathways are thus distinct. Activation of the Apo-2L pathway can, however, override the FAK survival signal, as others have shown that the large prodomain caspases cleave FAK.

Another Partner for Talin

Members of the band 4.1 family connect the actin cyto-

skeleton to transmembrane proteins that are exposed to the outside world. Talin is a perfect example: at focal contacts, it links actin to integrins. But the actin–talin–integrin link is mediated primarily by the carboxy-terminal fragment of talin, whereas most band 4.1 family members use their similar amino-terminal domains to bind transmembrane proteins. Also, the integrin binding does not explain how talin functions in cell migration or what localizes it to ruffles at the leading edge of migrating cells.

Using a two-hybrid scheme, Borowsky and Hynes find a talin-binding protein they name layilin (page 429). This transmembrane protein binds the amino terminus of talin, colocalizes with it at ruffles, and may bind extracellular carbohydrates via its C-type lectin domain. Borowsky and Hynes speculate that this early adhesive event may be dynamic, reminiscent of the transient binding of selectins as leukocytes roll over endothelial cells. The transient link could then mature to the more static talin–integrin connection at focal contacts.

Releasing Splicing Factors

On page 297, Misteli et al. lend support to the idea that phosphorylation controls the cycling of splicing factors from storage/reassembly sites to transcription sites. They look at SR proteins—factors important in both constitutive and regulated splicing. Although SR proteins are spread throughout the nucleoplasm, they are concentrated in speckles. These sites are made up of interchromatin granule clusters (IGCs), which are not sites of transcription, and perichromatin fibers (PFs), which emanate from the IGCs and do show transcriptional activity. SR proteins are recruited from IGCs to PFs when transcription is switched on.

SR proteins are characterized by one or two RNA recognition motifs (RRMs) that are needed to bind target mRNA, and a serine/arginine-rich (RS) domain. Misteli et al. find that deletion of either domain results in a failure of SR protein movement to transcription sites. SR proteins lacking an RRM leave the IGCs but do not find their targets, ending up in a diffuse distribution. But SR proteins lacking an RS domain, or with the serines replaced with glycines, never leave the IGCs. Release from the IGCs by serine phosphorylation could be mediated by any one of a number of kinases that have been shown to phosphorylate splicing factors and affect their distribution.

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