Rabs make their way to mitochondria

The large family of Rab GTPases has various functions, including the assembly of proteins required for endocytosis and membrane trafficking. Now, a new function for a Rab is emerging. On page 659, Alto et al. describe a Rab that affects mitochondrial fission. This GTPase, Rab32, also has an unusual binding partner, the cAMP-dependent kinase PKA.

The authors originally sought to identify novel AKAPs, proteins that anchor PKA to subcellular domains. They found Rab32, the first Ras family member GTPase shown to interact directly with PKA. Although a functional connection between the two has yet to be shown, association of the enzymes may facilitate localization of signal transduction pathways. The group is currently addressing this possibility by looking for other proteins that may interact with the complex.

After confirming that Rab32 was an AKAP, Alto et al. were surprised to find that it localized to mitochondria, where PKA is known to phosphorylate several proteins, including those involved in apoptotic pathways. Rab32’s function there seems to be separate from its PKA-binding activity, however, as mitochondria were not affected by Rab32 mutations that abolished this binding. In contrast, overexpression of a dominant–negative version of Rab32 lacking its GTP-binding activity resulted in an aggregation of elongated mitochondria around the microtubule organizing center. Thus, Rab32 activity may be required for mitochondrial fission. This suggests that Rab32 function is similar to that of other Rab proteins in trafficking, where they help assemble complexes required for fusion and fission.

PECAM-1 senses the pressure

Cells can sense when the pressure is on; they respond to fluid shear stress (FSS) by MAPK activation and reorganization of the actin cytoskeleton, for example. Although signaling cascades controlling these changes are well studied, the molecules that sense mechanical stimuli at the plasma membrane are generally unknown. On page 773, Osawa et al. present strong evidence that the adhesion molecule PECAM-1 acts as a mechanosensor and may be a model for other mechanical sensors.

PECAM-1 in endothelial cells is known to be phosphorylated rapidly upon sensing changes in FSS. PECAM-1 can then bind to and activate the SHP-2 phosphatase, which dephosphorylates and then falls off PECAM-1. Extracellular signal-related kinase (ERK) is also phosphorylated via RAS signaling under these conditions, activating several genes involved in arteriosclerosis. Osawa et al. now show that ERK activation depends on PECAM-1 tyrosine phosphorylation in direct response to mechanical stress.

Phosphorylation was triggered by tugging specifically on PECAM-1, using magnetic beads attached to anti–PECAM-1 antibodies. PECAM-1 molecules are attached to each other on the extracellular side; on the intracellular side, they may be attached to actin via catenin. This double tethering may trap the PECAM-1 molecules so that they cannot move easily in a flexing membrane, leading to distortion and exposure of the tyrosine residues to a nearby kinase.

Quantity assurance

The 2 micron plasmid persists at an optimal copy number of 60 per cell by replicating in conjunction with the host chromosomes only once per cell cycle. This limited replication requires a high fidelity system to divide plasmids evenly between daughter cells. Results from Mehta et al. on page 625 suggest that the plasmid accomplishes this by recruiting cohesin. The authors first noticed that mutations that disturb chromosome segregation similarly affect plasmid segregation, so they examined whether proteins involved in sister chromatid pairing also function during plasmid partitioning. They found that a cohesin subunit, Mcd1, was recruited by plasmid proteins Rep1p and Rep2p partitioning. They found that a cohesin subunit, Mcd1, in sister chromatid pairing also function during plasmid segregation, so they examined whether proteins involved chromosomes only once per cell cycle. This limited replication requires a high fidelity system to divide plasmids evenly between daughter cells. Results from Mehta et al. on page 625 suggest that the plasmid accomplishes this by recruiting cohesin. The authors first noticed that mutations that disturb chromosome segregation similarly affect plasmid segregation, so they examined whether proteins involved in sister chromatid pairing also function during plasmid partitioning. They found that a cohesin subunit, Mcd1, was recruited by plasmid proteins Rep1p and Rep2p to a plasmid sequence, STB. STB does not resemble chromosomal cohesin-binding sequences, and cohesin abnormally expressed during G1 was found on STB but not chromosomes. Thus, the plasmid has independently evolved the ability to recruit cohesin.

A noncleavable form of cohesin blocked segregation of the plasmids, suggesting that cohesin is used to ensure even partitioning of the plasmids. However, it is also possible that the effect is due to association of the plasmids with missegregating chromosomes, as the Rep proteins were seen in association with chromosomes. Mutations that perturb the ability of plasmids, but not chromosomes, to recruit cohesin should clarify these possibilities.
ABU to perform under stress

A report on page 639 identifies a family of proteins that are important when worms are unable to deal with ER stress. The results from Urano et al. suggest that these proteins are receptors for incorrectly folded proteins at the ER.

In worms and other eukaryotes, conditions that stress the ER, such as those that inhibit glycosylation, activate the unfolded protein response (UPR). The UPR comprises several signaling pathways that increase the protein folding capacity of the ER through the induction of chaperones and other protein processing and trafficking components.

The UPR does not, however, explain everything. Using microarrays, Urano et al. identified a default pathway activated in worms under ER stress when the UPR is blocked. Among the genes identified were nine members of a family they named *abu*, for activated in blocked UPR. RNAi inhibition of one or more ABU proteins in UPR-deficient mutants severely impaired their survival during ER stress. In wild-type worms, interference with ABU function by RNAi induced ER stress genes, indicating that the ABU proteins may also function under nonstress conditions.

Localization experiments indicated that ABU-1 is an ER-localized integral membrane protein. The ABU family resembles mammalian scavenger receptors, plasma membrane proteins that bind extracellular proteins that are oxidized or otherwise chemically modified. ABU proteins may serve a similar function at the ER, although a direct interaction with improperly folded proteins has yet to be shown. Mammals do not have obvious orthologues of ABU proteins. But recent results showing that the ER and plasma membrane may fuse during phagocytosis suggest that the distinction between plasma membrane proteins and ER proteins may not be as clear as previously suggested. Thus, the scavenger receptors may be able to fill in for ABU proteins in the ER.

Selectin flexible cells

Leukocytes use glycoprotein ligands to grab onto selectins on blood vessel walls and roll along with the blood flow. The longer the cell can roll without detaching, the greater its chances are of responding to chemotactic signals telling it to traverse the endothelium at a site of inflammation. Now, on page 787, Yago et al. show that flexibility helps a cell avoid detachment when circulatory forces are high.

As shear force increases with higher blood pressure and smaller vessel size, leukocytes can maintain a stable rolling velocity, an ability known as an automatic braking system (ABS). Artificial microspheres containing certain ligands also roll on selectins, but lack an ABS—under high forces, their rolling velocity increases, and they are more apt to detach. Recent discussion in the field has centered on whether this difference reflects a problem with ligands or their carriers: either the microsphere ligands may not be optimal for binding under high forces, or the microspheres may lack some structural feature of cells.

Yago et al. address this issue by using the same ligands on both microspheres and a hematopoietic cell type that does not express selectin ligands. Their results show that ABS is a function of the ability of the cell to change its shape, a feature not found in rigid microspheres. Cells made more flexible by depolymerizing actin rolled more efficiently. Making cells stiffer by fixation or addition of a cholesterol chelator made cells roll more like microspheres. ABS efficiency may be improved through the flattening of a cell, placing more of its ligands in contact with selectin on endothelial cells. Rolling leukocytes are also known to extend long thin tethers at their trailing edge; these structures may reduce the force exerted on the rest of the cell.