Making Mitochondria

Fission or Fusion?

Readers scanning this issue could be excused for thinking they had picked up the Journal of Mitochondrial Biology. Three papers report on new components involved in fission of the budding yeast mitochondrial outer membrane. A fourth paper is an investigation into the function of Mgm1p, a known mitochondrial protein from budding yeast. All the papers are discussed in more detail by van der Bliek (page F1).

Mgm1p or its homologues have been reported to be localized to the mitochondrial outer membrane (in budding yeast) or mitochondrial matrix (in fission yeast). Wong et al. report, based on protease sensitivity and immunogold labeling, that Mgm1p is a peripheral membrane protein that is bound to the mitochondrial inner membrane and exposed to the intermembrane space (page 341). Mgm1p is similar to dynamin, a protein involved in pinching off endocytic vesicles, so in theory Mgm1p could be involved in fission of the mitochondrial inner membrane. Its outer membrane counterpart appears to be Dnm1p, another dynamin-like protein.

But the mutant phenotypes do not lead directly to this simple interpretation. Wong et al. report that loss of Mgm1p leads to mitochondrial fragmentation (not fusion, as with loss of Dnm1p) immediately after temperature upshift of mgm1Δ cells. The previously reported mgm1Δ phenotype (aggregated mitochondria with mitochondrial DNA loss) comes only later.

Similar mitochondrial fragmentation has been reported after loss of the fusion protein Fzo1p. But cells lacking Mgm1p can still undergo Fzo1p-dependent fusion if Dnm1p is absent. Wong et al. suggest that the outer membrane apparatus detects defects arising from the loss of Mgm1p, and responds with overactive fission. Mgm1p’s normal function may be to form either or both of the fission sites or infoldings (cristae) of the inner mitochondrial membrane.

Adding to the Fission Apparatus

Fekkes et al. (page 333), Tieu and Nunnari (page 353), and Mozdy et al. (page 367), have identified proteins required for fission of the mitochondrial outer membrane. All three groups looked for suppressors of mutants with excessive mitochondrial fission, although Fekkes et al. started with a mutant lacking Mgm1p, whereas the other two groups started with mutants lacking Fzo1p.

The three groups each recovered three complementation groups, including multiple alleles of DNM1, which encodes a known dynamin-like protein. Fekkes et al. and Tieu and Nunnari focus on Gag3p/Mdv1p. This peripheral membrane protein is associated with the mitochondrial outer membrane, and contains WD repeats, which usually mediate protein–protein interactions. The protein binds to Dnm1p in a two-hybrid assay, although not in a coimmunoprecipitation assay. Tieu and Nunnari find that Gag3p/Mdv1p colocalizes with Dnm1p in punctate structures at sites of fusion. In the absence of Dnm1p, some Gag3p/Mdv1p is associated with mitochondria, but it is no longer in punctate structures.

Mozdy et al. concentrate on Fis1p, which is localized all over the mitochondrial surface and spans the mitochondrial outer membrane. As the first transmembrane protein to be implicated in a dynamin-related fission event, Fis1p may be important in targeting the fission apparatus to the correct site. Mozdy et al. and Tieu and Nunnari find that, without Fis1p, some Dnm1p and Gag3p/Mdv1p still find their way to mitochondria, although they end up in just a few large clumps. Without both Fis1p and Dnm1p, mitochondrial localization of Gag3p/Mdv1p is lost completely. Meanwhile, Fekkes et al. find that the as-yet uncloned Gag2p is important for the localization of Dnm1p to the mitochondrial surface.

Fis1p may both distribute and activate the Dnm1p complex, with Gag3p/Mdv1p making a later, Dnm1p-dependent appearance. Gag3p/Mdv1p presumably brings in other components, perhaps including the product of a fourth complementation group more recently isolated by the Nunnari group.

Fusion Requirements

Continuing with the membrane theme, Melikyan et al. investigate membrane fusion during entry of HIV into the cell (page 413). They conclude that it is the formation of a six-helix bundle in the HIV Env protein that drives fusion, not the presence of a completely formed bundle structure. As Doms and Moore explain in an accompanying Comment (page F9), this is akin to catching fish thanks to the motion of a harpoon, rather than the voluntary impaling of fish onto a stationary harpoon.

After HIV binds the cell’s receptors and coreceptors, trimers of the gp41 portion of Env insert three fusion peptides into the target cell membrane. Next, gp41 bends back onto itself to form the six-helix bundle, bringing the NH2-terminal fusion peptides and COOH-terminal transmembrane domains close together. These protein regions are embedded in op-
posite membranes. Bundle formation brings them so close together, according to Melikyan et al., that membrane fusion occurs simultaneously.

Bundle formation is known to be required for fusion, as the T20 and T21 peptide inhibitors block both events by mimicking the gp41 self-association. But perhaps bundle formation occurs before fusion. Melikyan et al. try to trap a hypothetical intermediate that is bundled, but not yet fused. They first pause the reaction at 23°C (after receptor binding, but just before fusion), and then make a quick jump to 37°C. But, at least within the time resolution of this assay, a bundled-but-not-fused intermediate cannot be trapped by T20 after a rapid return to 4°C. Melikyan et al. further demonstrate the bundling–fusion interdependence by showing that without fusion (in the presence of a membrane-curving lipid) there can be no six-helix formation.

Fusion mediated by influenza hemagglutinin (HA) follows a structural logic similar to that of gp41-mediated fusion. Armstrong et al. (page 425) find that HA requires a transmembrane domain of 17 amino acids or more for full fusion activity. This suggests that the protein must span the membrane so that during any conformational change the protein is not ripped out of the viral membrane. Instead, the protein must be correctly positioned so that it can break the so-called hemifusion diaphragm (in which only one leaflet of each membrane has mixed) to create a fusion pore.

Fusion mediated by SNARE proteins during exocytosis may operate in a similar way. Grote et al. (page 453) replace the transmembrane domains of exocytic SNARE proteins with lipid-anchoring domains, and report a dominant inhibition of membrane fusion that may resemble the fusion failure seen with the truncated HA proteins.

**Autophagy Gets Modified**

Starved cells turn on a drastic shutdown process called autophagy, in which double-membrane structures (autophagosomes) engulf large portions of the cytoplasm and destroy everything within. Budding yeast *apg* mutants fail to turn on this program, and some of the Apg proteins have been shown to undergo a ubiquitin-like covalent modification. Now, Kirisako et al. (page 263) report another Apg covalent modification system that may be involved in autophagosome formation.

Kirisako et al. originally observed that their myc-tagged Apg8p kept on losing its COOH-terminal tag. Purified Apg4p can reproduce this cleavage in vitro, which exposes a glycine residue. The glycine is needed for binding of Apg6p to membranes, apparently because Apg8p undergoes a covalent modification with an unidentified molecule. The modification does not take place in cells that lack Apg7p, an enzyme with similarities to E1 ubiquitination enzymes.

The modified Apg8p is released from membranes by a second cleavage by Apg4p. Kirisako et al. suggest that modified Apg8p, which is present on premature autophagosomes, may bring in components necessary for autophagosome assembly before it is subsequently removed by Apg4p.

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