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SPOTLIGHT

Mitochondrial double membrane fission: A mystery solved?

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It has long been an unresolved question whether the division machineries that assemble on the mitochondrial surface cooperate with factors inside the organelle. Now, two studies by Connor et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202303147) and Fukuda et al. (2023. *Mol. Cell.* https://doi.org/10.1016/j.molcel.2023.04.022) have identified an intermembrane space protein that is crucial for mitochondrial double membrane division.

The elaborate architecture of eukaryotic cells is determined by the shape and positioning of membrane-bound organelles, which are constantly remodeled during cell division, differentiation, and in response to ever-changing physiological conditions. Mitochondria are highly dynamic organelles as well. Their fusion and fission is important, for example, for their inheritance during cell division, for the production and distribution of metabolic energy, and during apoptosis (1).

Dynamin-like proteins (DLPs) are large GTPases that mediate fission and fusion of cellular membranes. Dynamin, the founding member of this protein family, forms a helical polymer that assembles around the neck of a budding vesicle during endocytosis. It harnesses the energy of GTP hydrolysis to sever the plasma membrane and release the newly formed vesicle into the cytoplasm (2). The division of mitochondria proceeds in a very similar way. A dynamin-related protein, DRP1 in mammals and Dnm1 in yeast, is recruited by receptor and adaptor proteins to the surface of the outer membrane, where it assembles into a helical structure surrounding the organelle. Constriction of this ring powered by GTP hydrolysis then drives the division of the organelle (3).

In contrast to classical dynamins, DLPs dividing mitochondria have to sever two membranes. For a long time, it has been an open question whether both mitochondrial membranes are divided simultaneously, or whether additional proteins exist to support inner membrane division (1, 3). Now, a paper in this issue of *JCB* (4) and a related study recently published in *Molecular Cell* (5) report the identification of a novel protein that contributes to mitochondrial fission by acting from inside the organelle.

Both groups used the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* as model organisms to study mitochondrial dynamics. The ORF *YIL156W-B* encodes a small protein of 73 amino acids in *S. cerevisiae*. A previous proteomic study revealed the localization of this protein in the mitochondrial intermembrane space (IMS), but its function remained unknown (6). Connor et al. (4) observed that deletion mutants have net-like mitochondria strikingly resembling mutants lacking Dnm1. These characteristic nets are formed by ongoing mitochondrial fusion that is not balanced by mitochondrial fission (7). The authors further provide genetic evidence for a role of this protein in mitochondrial fission and show that it assembles into punctate structures that frequently co-localize with Dnm1. Therefore, they named it Mdi1 (Mitochondrial Division IMS 1).

Damaged or surplus mitochondria are removed from the cell by mitophagy, i.e., selective autophagic degradation of mitochondria. This process requires that a mitochondrial fragment small enough to be engulfed by the phagophore is split off from the mitochondrial network. Fukuda et al. (5) found that mitophagy is blocked in mutants lacking a protein that they named Atg44 (Autophagy related 44). Upon induction of mitophagy, mutant cells show mitochondrial protrusions that are produced by the autophagy machinery but remain connected to the main body of the mitochondrion because mitochondrial fission is blocked. Atg44 is identical to Mdi1—in the following, I adopt the name "mitofissin" that was coined for this new protein family with members in fungi, algae, and some plants (5).

What might be the molecular function of mitofissin? Both secondary structure prediction (4) and crystal structure analysis (5) show that mitofissin contains α helices that form amphipathic structures that could interact with lipid membranes. Indeed, Fukuda et al. (5) show that mitofissin binds to lipid membranes in vitro, preferentially at sites with high positive curvature, and is able to mediate fission of lipid nanotubes. Connor et al. (4) demonstrate that mutations that disrupt the amphipathic nature of the second predicted α helix abolish the protein's function. Together, these observations support a model in which hydrophobic residues exposed on the surface of mitofissin interact with the mitochondrial inner and/or outer membrane and thereby locally

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distort the lipid bilayer to support or even mediate fission of the membrane(s) (4, 5).

While both studies agree on a role of mitofissin in inducing membrane fragility, they reach different conclusions regarding the dependence of this activity on Dnm1. Connor et al. (4) report that loss of mitofissin and Dnm1 produce very similar phenotypes and genetic interactions and that both proteins partially co-localize. These phenotypes are independent of mitophagy and argue for a general role of mitofissin in mitochondrial division. Therefore, these authors propose that both Dnm1 and mitofissin are indispensable for completion of mitochondrial double membrane fission.

On the other hand, it has been shown earlier that Dnm1 is dispensable for mitochondrial division that occurs during mitophagy to allow engulfment of mitochondrial fragments by the phagophore (8). Consequently, Fukuda et al. (5) observed that overexpressed mitofissin is able to induce mitochondrial fragmentation in Dnm1-deficient cells, and purified mitofissin mediates fragmentation of lipid nanotubes in vitro without requiring any other factors. These are indications that mitofissin activity does not require Dnm1. Therefore, these authors propose that mitofissin is specifically required for Dnm1-independent mitochondrial fission during mitophagy, rather than Dnm1-dependent fission under homeostatic conditions.

How can these apparently contradicting views be reconciled? Several observations in the literature indicate that mitochondrial division can occur in the absence of Dnm1. This is not only true for mitophagy (8), but also for the fragmentation of mitochondria during meiosis and ascospore formation (9) and during cytokinesis of cells lacking Dnm1 (10). It is conceivable that an external force squeezing the mitochondrial outer membrane at the site selected for division is essential for scission of the mitochondrial double membrane. This can be exerted either by the Dnm1 ring under homeostatic conditions, or by phagophore closure in mitophagy, or by the contractile ring at the bud neck during cytokinesis. However, this external force frequently may not be sufficient to complete division of both membranes. Thus, destabilization of the mitochondrial double membrane by mitofissin may be crucial to allow efficient division of the organelle in several Dnm1-dependent and Dnm1-independent fission scenarios.

Certainly, more work will be required to test this hypothesis and mechanistically further dissect the molecular role of mitofissin. Also, it will be interesting to see in the future whether functionally equivalent proteins exist to support mitochondrial fission in human cells.

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