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Coupling mitochondrial and cell division

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Abstract

The mitochondrial network fragments during mitosis to allow proper segregation of the organelles between daughter cells. Two mitotic kinases, the cyclin B–CDK1 complex and Aurora A, are now shown to cooperate with the small G protein RALA and its effector RALBP1 to promote DRP1 phosphorylation and mitochondrial fission.

Mitochondria, the powerhouses of the eukaryotic cell, acquire intriguing shapes and frequently change their morphology by shifting the balance between fusion and fission in response to the cellular environment and a multitude of signals. Even in cells under stasis, mitochondria rapidly cycle through rounds of fission and fusion. Thus, the balance of these opposing events is accurately controlled to maintain the overall architecture and activities of mitochondria¹. Mitochondria have a distinct outer membrane surrounding an inner membrane that folds into cristae containing the oxidative phosphorylation machinery, and division requires scission of both of these membranes. Dynamin-related large GTPases, which are critical for dynamic regulation of mitochondrial morphology, are found on these different membranes: DRP1 mediates fission and is found in the cytosol and docked to the outer membrane; mitofusins (MFN1 and MFN2) mediate fusion and are integrally embedded in the outer membrane with the GTPase domains facing the cytosol; and OPA1 mediates fusion, localizes in the intermembrane space and is attached to the inner membrane. The loss of balance between fusion/fission is linked to mitochondrial and cellular dysfunctions including apoptosis and neurodegeneration. Indeed, mutations in *MFN2* and *OPA1* have been reported in patients with Charcot Marie Tooth neuropathy type 2A and dominant optic atrophy type I, respectively. Although both mitochondrial fusion and fission are clearly essential for animal survival as discerned from knockout mouse models^{2–4}, the molecular or cellular processes that are affected by mitochondrial dynamics is not yet resolved. One important role seems to be to facilitate the even distribution of organelles between daughter cells during mitosis. On page 1108 of this issue, Kashatus *et al.* add an interesting new dimension to our understanding of how mitochondrial fission is regulated during mitosis through DRP1 phosphorylation⁹.

DRP1 is a large GTPase protein that is conserved from yeast to man, and mediates scission of both outer and inner membranes. DRP1 proteins constrict the outer mitochondrial

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membrane at division sites after assembling into spiral complexes⁵, which appear as punctate foci when visualized by light microscopy⁶. Conformational changes in DRP1 induced by GTP hydrolysis mediate membrane constriction and/ or scission processes. Although other proteins responsible for the regulation of mitochondrial morphology, including MFN1/2 and OPA1, and potential DRP1 receptors, such as MFF, MiD49, MIEF1 (also known as MiD51) and FIS1, are integrated into mitochondrial membranes through transmembrane domains, most DRP1 is localized in the cytosol. Only a small fraction (~3%) of DRP1 is associated with the surface of the mitochondrial outer membrane under normal conditions⁷. Therefore, mitochondrial fission is thought to start with DRP1 accumulation from the cytosol onto prospective mitochondria division sites. Regulation of DRP1 itself and/or its mitochondrial receptors by post-translational modifications that result in higher order conformational changes may be important for the cycling of DRP1 on and off mitochondria.

The first report of post-translational modification of DRP1 identified cyclin B–Cdk1-mediated phosphorylation during mitosis. Taguchi *et al.* elegantly demonstrated, using cell-cycle-synchronized HeLa cells, that mitochondria become fragmented in the early mitotic shift from prophase to metaphase when DRP1 is phosphorylated by cyclin B–CDK1, and then re-form long tubular structures after completion of cell division (interphase)⁸. Mitochondria have to be judiciously distributed from mother cells to daughter cells. Thus, proliferation followed by segregation of the organelle during mitosis seems to be important for organism survival and needs to be carefully regulated. Although mitochondrial distribution is well studied by both genetic and biochemical approaches in the budding yeast *Saccharomyces cerevisiae*, investigations of mitochondrial morphology regulation in mammalian cells throughout the cell cycle have been lagging.

Kashatus *et al.* provide fresh insight into this important process by demonstrating that the mitotic kinase Aurora A and the small Ras-like GTPase RALA control the recruitment of DRP1 to mitochondria. Aurora A is known as a serine/threonine kinase that is highly conserved from yeast to man, and has a pivotal role in many aspects of cell division, such as mitotic entry, chromosome segregation and spindle assembly¹⁰. A number of studies indicate that mutation or disruption of the Aurora A gene causes mitotic abnormalities in various species. On the other hand, RALA, a small G protein belonging to the Ras superfamily, controls and participates in cellular processes such as vesicle sorting, cell morphology and gene expression by cycling between active GTP-bound and inactive GDP-bound conformations¹¹. Based on their previous finding that Aurora A phosphorylates RALA and alters its subcellular location from the plasma membrane to an internal membrane¹², Kashatus *et al.* found that phosphorylated RALA, as well as its phosphomimetic mutant, accumulate preferentially on mitochondria. They also demonstrate that DRP1 protein levels in the mitochondrial fraction are notably reduced both in cells with diminished levels of RALA and in cells overexpressing a kinase-inactive mutant of Aurora A, which, in parallel, inhibits mitochondrial fission and induces a more highly interconnected mitochondrial network.

But how do these proteins affect the recruitment of DRP1? A hint to the answer came from the analysis of RALBP1, a multifunctional effector of RALA. By immunoprecipitation

analysis of a mitochondrial-rich fraction prepared from mitotic cell extracts of synchronized cells, the authors found that RALBP1 and cyclinB–Cdk1 interact with each other and phosphorylated DRP1 (cyclin B–Cdk1 were previously shown to phosphorylate DRP1 in mitosis⁸). In addition, *in vitro* kinase assays show that RALBP1 stimulates cyclin B–Cdk1 kinase activity. These findings suggest that RALBP1, following recruitment to mitochondria by RALA, may form a mitosis-specific complex with cyclin B–CDK1, and that this interaction potentiates cyclin B–Cdk1 kinase activity towards DRP1 and promotes DRP1 oligomerization and subsequent mitochondrial fission (Fig. 1). Time-lapse video microscopy to observe mitochondrial dynamics during cell cycle progression clearly shows that knockdown of either *RALA* or *RALBP1* inhibits mitochondrial fission. Intriguingly, following knockdown of *RALA* or *RALBP1*, mitochondria are elongated even during telophase in some cells, and some of these mitochondria then seem to get stuck in the cleavage furrow as if they are strangled by the contractile ring. Some cells also fail to distribute mitochondria equally between daughter cells, resulting in reduced ATP levels in daughter cells, or worse, a decrease in the number of metabolically active cells. Kashatus *et al.* cast a spotlight on mitotic mitochondrial fission during mitosis and bring Aurora A, RALA and RALBP1 to the scene.

However, many questions remain. It remains unclear, for example, whether the phosphorylation of DRP1 is always essential for membrane scission. If so, cyclin B–CDK1 can phosphorylate DRP1 during mitosis, but another kinase would be required for steady-state fission activity of DRP1 during interphase. If not, we need to ascertain how unphosphorylated DRP1 is recruited to the specific positions on the mitochondria and how phosphorylation increases this steady-state activity. Furthermore, cyclic-AMP-dependent protein kinase (PKA) has been identified to phosphorylate a different but nearby serine residue during starvation^{13–15}. Unlike cyclin B–CDK1, surprisingly, PKA-mediated phosphorylation has the opposite function of inhibiting the translocation of DRP1 to mitochondria. The molecular mechanism whereby nearby phosphorylation events mediate opposing effects on DRP1 activity will be important to elucidate. A growing body of research indicates that mitochondrial division responds to cellular stimuli in highly regulated ways where a central node is the modulation of DRP1, not only by phosphorylation, but also by other post-translational modifications including ubiquitylation, SUMOylation and S-nitrosylation. How the supporting cast members, MFF, MiD49, MIEF1 and FIS1, fit into the scene to help the central player of mitochondrial fission, DRP1, also remains a mystery.

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Figure 1.

Mitosis-specific mitochondrial fission involves the phosphorylation of DRP1. Mitochondrial morphology is coordinated with the cell cycle. During metaphase, in which condensed chromosomes align in the middle of the cell, mitotic kinase Aurora A phosphorylates RALA, which relocates to mitochondria. This encourages the formation of a complex consisting of phosphorylated RALA, RALBP1 and cyclin B–CDK1 on mitochondria, and mediates the phosphorylation of DRP1. Finally, oligomeric DRP1 wraps around the mitochondrial surface and mitochondrial division ensues.