Mitochondrial lipid transport and biosynthesis: A complex balance

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Little is known about how mitochondrial lipids reach inner membrane–localized metabolic enzymes for phosphatidylethanolamine synthesis. Aaltonen et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201602007) and Miyata et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201601082) now report roles for two mitochondrial complexes, Ups2–Mdm35 and mitochondrial contact site and cristae organizing system, in the biosynthesis and transport of mitochondrial lipids.

Mitochondria are dynamic organelles involved in various cellular processes, including energy production, cell cycle, and apoptotic signaling. Mitochondria also have an important role in cellular lipid homeostasis because they orchestrate the synthesis of some key membrane phospholipids, such as phosphatidylethanolamine (PE) and phosphatidylcholine (PC; Clancey et al., 1993; Trotter et al., 1993). These double membrane–bound organelles do not participate in vesicle trafficking processes but obtain most of their lipids from the ER through membrane contact sites called mitochondrial-associated ER membranes (Levine, 2004). In yeast, for instance, the well-studied ER–mitochondria encounter structure complex has the capacity to hold the ER and the mitochondrial outer membrane together and may govern lipid transfer at mitochondrial-associated ER membranes (Kornmann et al., 2009; AhYoung et al., 2015). The transfer of lipid precursors, such as phosphatidylserine (PS) and phosphatidic acid (PA), across the mitochondrial intermembrane space is also seemingly required for the synthesis of PE and of the (mitochondrion-specific) phospholipid cardiolipin (CL), respectively, by enzymes present only in the mitochondrial inner membrane.

High levels of PE and CL are produced and maintained in mitochondrial membranes and are required for important mitochondrial functions, including cristae development and the stabilization of respiratory complexes (Böttinger et al., 2012). Recent work has demonstrated that deficient intramitochondrial transport of PA alters these functions and mitochondrial morphology because of reduced CL levels (Potting et al., 2013). The key phospholipid PE is produced from PS by the inner membrane–resident enzyme PS decarboxylase 1 (Psd1) and can be exported to the ER for further conversion to PC (Vance, 2015). However, extramitochondrial PE synthesis cannot fulfill mitochondrial functional integrity, pointing to an important role for the Psd1 pathway in membrane lipid homeostasis. This functional dependence is illustrated by the embryonic lethality of mice that lack mitochondrial PE synthesis (Steenbergen et al., 2005).

How lipids reach the metabolic enzymes located in the mitochondrial inner membrane is largely unknown. However, highly conserved proteins located in the mitochondrial intermembrane space from the Ups family in yeast (PRELID in human) have been identified as key players in phospholipid metabolism (Osman et al., 2009; Tamura et al., 2009). Ups1 and Ups2 are intrinsically unstable, but form heterodimeric complexes with the Mdm35 protein (human TRIAP1) that prevent them from being degraded by mitochondrial proteases (Tamura et al., 2010). Connerth et al. (2012) have uncovered a role for the Ups1–Mdm35 complex as a lipid transfer device that can shuttle PA from the outer to the inner membrane, where it is converted to CL. In contrast, the exact function of Ups2 remains unknown. Although a Ups2–Mdm35 complex seems to be required for maintaining proper levels of PE in mitochondrial membranes, the dependence of PE synthesis on Ups2 has recently been thrown into question (Osman et al., 2009; Tamura et al., 2009). In two new studies, Miyata et al. and Aaltonen et al. demonstrate that Ups2–Mdm35 acts as a lipid shuttling complex that mediates the transport of PS between mitochondrial membranes for the production of PE by Psd1 (Fig. 1). Interestingly, however, both groups propose that Ups2–Mdm35 is dispensable for mitochondrial PE synthesis in certain situations and is compensated for by another mechanism.

By creating an assortment of mutant yeast strains that lack pathways for PE metabolism in combination with *ups2*Δ, Miyata et al. (2016) reveal that Ups2 is specifically implicated in Psd1-dependant PE synthesis. In the other study, Aaltonen et al. (2016) demonstrate that PE levels are restored in *ups2*Δ cells that express the human protein SLMO2 (also termed PRELID3B), thereby defining the functional human orthologue of Ups2. To characterize the function of Ups2–Mdm35 both groups reconstituted lipid transfer reactions in vitro using artificial membranes (liposomes). They show that the purified Ups2–Mdm35 complex can transfer PS between liposomes. Interestingly, the incorporation of CL in these membranes promoted robust PS transfer (Aaltonen et al., 2016), suggesting a possible interdependence between several lipid-transfer reactions in the mitochondrial intermembrane space (Fig. 1). Ups–Mdm35, START, and phosphatidylinositol-transfer protein domains

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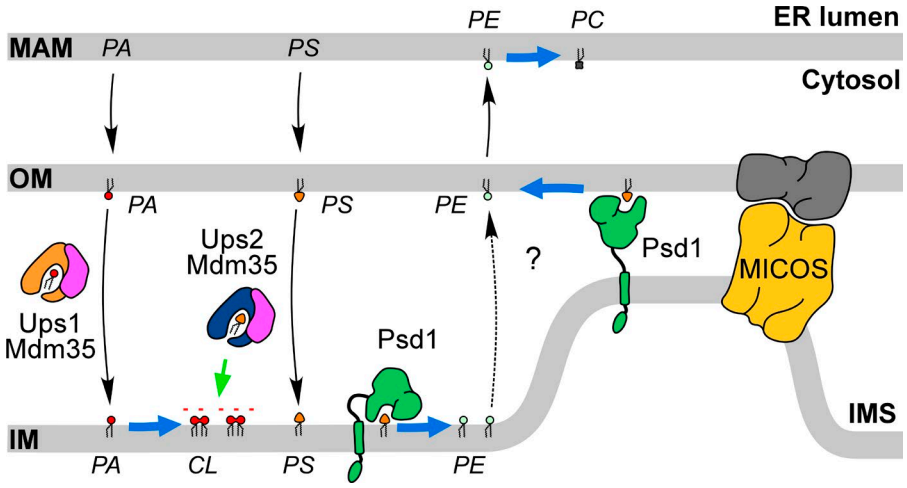

Figure 1. **Mitochondrial phospholipid trafficking and metabolism organized by Ups–Mdm35 complexes and MICOS.** Ups1–Mdm35 and Ups2–Mdm35 transport PA and PS, respectively, from the outer (OM) to the inner (IM) membrane of mitochondria. PA is then converted to CL, which helps, together with other negatively charged lipids, to bind Ups2 to the membrane and to deliver PS. PS is subsequently converted to PE by the IM-resident enzyme Psd1. MICOS-mediated membrane contact sites might facilitate PE synthesis directly at the OM to allow for PE export and its subsequent conversion to PC at the ER. Lipid conversions are denoted by blue arrows. IMS, mitochondrial intermembrane space; MAM, mitochondrial-associated ER membrane.

share remarkable structural similarities that hint at similar mechanisms of action (Watanabe et al., 2015). Consistently, Ups2–Mdm35 cannot execute PS transfer upon deletion of the Ω1 loop in Ups2, which corresponds to the lid for the lipid-binding pocket of several lipid-transfer proteins (Miyata et al., 2016). Note that both groups present contradicting findings on the capacity of Ups2–Mdm35 to transport PA between liposomes. However, the loss of Ups2 did not lead to a decrease in CL in vivo, but retarded the conversion of PS to PE, indicating that Ups2–Mdm35 is involved in transporting PS to the site of PE synthesis in mitochondria.

Surprisingly, the Ups2–Mdm35 complex is not always active in supporting mitochondrial PE synthesis because it is subjected to yeast homeostatic regulation in response to environmental change (Miyata et al., 2016). Indeed, by using pulse-chase experiments with radiolabeled L-serine, both groups noticed that the loss of Ups2 only slightly affected the accumulation of PE in mitochondria of cells growing logarithmically in glucose-containing medium. In contrast, Miyata et al. (2016) show that Ups2 greatly enhanced PS conversion to PE in mitochondria shifted into the respiration-active state—a condition that occurs when glucose is exhausted or when yeast are cultivated with nonfermentable carbon sources. They demonstrate that changing yeast medium from fermentable to nonfermentable conditions consistently increased Ups2 abundance by up to threefold and that a peak in Ups2 expression occurred exactly at the diauxic shift, when yeast metabolism switches from glycolysis to respiration. Although this work highlights the importance of Ups2 in respiration-active mitochondria and at the diauxic shift, Miyata et al. (2016) note that the loss of Ups2 does not fully eliminate the conversion of PS into PE in mitochondria, even in nonfermentable conditions, suggesting the existence of another mechanism for Psd1-mediated PE synthesis.

Aaltonen et al. (2016) further investigate how Psd1 carries out PE synthesis in the absence of PS transfer. They show that Psd1 reconstituted into liposomes displays measurable PS-decarboxylase activity in trans (i.e., when the lipid substrate and the enzyme are present in different membranes). If transposed to mitochondria, this mechanism suggests that the inner membrane–based Psd1 could exert its activity directly at the outer membrane, provided that both membranes are adequately juxtaposed (Fig. 1). Aaltonen et al. (2016) propose that this process is facilitated by the mitochondrial contact site and cristae-organizing system (MICOS), which brings the inner and outer membranes into close apposition (Pfanner et al., 2014). Consistently, the rate of Psd1-dependent PE synthesis was reduced in a yeast MICOS deletion mutant. Additional experiments revealed that when extramitochondrial PE synthesis is blocked an artificial tether protein that joins the inner and outer membrane together can restore the growth of MICOS-deficient cells. However, only a fraction of normal PE levels was restored and the artificial tether could not restore growth in nonfermentable carbon sources.

In the absence of MICOS, mitochondria have an altered ultrastructure and defects in cristae morphology, thus pointing to its key functions in membrane organization. Aaltonen et al. (2016) linked these functions to PE metabolism because loss of Ups2 rescued cristae morphogenesis in MICOS-deficient mitochondria. It is noteworthy that loss of Ups2 also partially rescues CL synthesis in Ups1-defective cells (Osman et al., 2009; Tamura et al., 2009). In contrast, loss of Ups1 does not rescue PE synthesis in *ups2*Δ cells (Miyata et al., 2016). The reason that Ups2 negatively affects mitochondrial morphology and CL synthesis remains to be determined. One hypothesis is that the Ups2–Mdm35 complex not only transports PS but also promotes the reverse transfer of PA from the inner to the outer membrane, thereby acting as a lipid exchanger akin to other PS-transfer proteins (Chung et al., 2015; Moser von Filseck et al., 2015). It is also unclear whether MICOS binds lipids and mediates their transfer directly, as is proposed for other tethering complexes (AhYoung et al., 2015). Such activity would explain why the artificial tether only partly rescues the phenotype of MICOS-deficient cells. Thus, key details of the MICOS function remain to be determined. Finally, it will be interesting to know whether Psd1 shifts its preference for acting in the trans or the cis orientation (i.e., when lipid transfer by Ups2–Mdm35 is active) according to the metabolic adjustments experienced by mitochondrial membranes.

It is now clear that mitochondrial membrane biogenesis depends on a delicate lipid balance that is tightly controlled by lipid transfer proteins of the Ups/PRELID family and by MICOS-mediated membrane contact sites. Future experiments are required to understand how these key mechanisms are coordinated, as some of them appear to be functionally interdependent but also highly regulated in response to metabolic demand.

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