Crystal Structures of the Ups1-Mdm35 Complex Reveal the Mechanism of Phospholipid Transfer in Mitochondria

Ups1, a conserved mitochondrial intermembrane-space protein, mediates phosphatidic acid (PA) transfer between the outer and inner mitochondrial membranes in cooperation with Mdm35. We determined the crystal structures of the Ups1-Mdm35 complex with and without PA. The Ups1-Mdm35 complex constitutes a single domain that has a deep pocket, which encloses a PA molecule, and a flexible Ω-loop lid. Structure-based mutational analyses revealed that basic residues at the bottom and near the entrance of the pocket and the lid of Ups1 play essential roles in its PA transfer activity and that dissociation of Mdm35 from Ups1 aids membrane binding and PA transfer.

Normal functions of mitochondria rely on optimal levels of phospholipids in the mitochondrial outer membrane (OM) and inner membrane (IM), including a mitochondrial signature phospholipid cardiolipin (CL). CL is synthesized through several steps of conversion and modification of phosphatidic acid (PA) by a chain of enzymes localized in the mitochondrial IM. Since PA is synthesized on the endoplasmic reticulum (ER) membrane, PA needs to be transported from the ER membrane to the mitochondrial IM by crossing the mitochondrial OM for CL biosynthesis.

Recently, Connerth et al. reported that Ups1, a yeast member of the conserved mitochondrial intermembrane space (IMS) proteins, mediates PA transfer between the mitochondrial OM and IM [1]. Ups1 is synthesized in the cytosol and imported into the IMS with the aid of Mdm35, a soluble protein in the IMS that forms a stable complex with Ups1 and assists the PA transfer by Ups1. However, the precise mechanisms of the PA transfer through the aqueous IMS by Ups1 and Mdm35 remained unclear.

To address this problem, we determined the crystal structure of the Ups1-Mdm35 complex at 1.40 Å resolution (**Fig. 1a**) [2]. Ups1 contains two α-helices and a seven-stranded antiparallel β-sheet, which is folded into a half-barrel structure, and has an Ω-loop formed between the β3 and β4 strands. Ups1 is structurally related to START (StAR-related lipid transfer) domains, which are found in several lipid transfer proteins [3, 4]. Mdm35 in the complex with Ups1 comprises an antiparallel α-hairpin and a C-terminal short α-helix. Mdm35 embraces Ups1 using the hydrophobic regions of the three α-helices. Thus, Ups1 and Mdm35 form a heterodimer as a single-domain-like structure. Like other START domains, Ups1 contains a positively charged deep pocket to which lipid molecules could bind. The entrance of the pocket is virtually closed by the flexible Ω-loop, suggesting that the Ω-loop may function as a lid that regulates lipid binding to the pocket. To further investigate the role of the pocket of Ups1 in the PA transfer, we determined the crystal structure of the Ups1-Mdm35 complex with DLPA (PA with 12:0-12:0 acyl chains) at 3.20 Å resolution (**Fig. 1b**). The bound DLPA molecule is completely enclosed in the pocket of Ups1, with the phosphate head positioned at the bottom of the pocket and the acyl-chain tails oriented toward the entrance of the pocket. On the basis of these structural characteristics, *in vitro* PA transfer activities of several Ups1 mutants were measured by a fluorescent-based lipid transfer assay. Interestingly, substitution of the basic residues at the bottom or near the entrance of the pocket with acidic glutamate residues or deletion of the Ω-loop lid abolished the PA transfer activities, suggesting that these basic residues and the Ω-loop lid play essential roles in the PA transfer activity of the Ups1-Mdm35 complex.

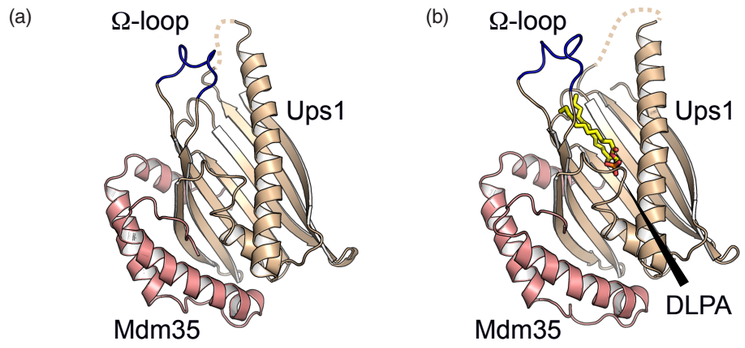

Figure 1: Crystal structures of the Ups1-Mdm35 complex as an apo form **(a)** and a DLPA-bound form **(b)**. Ups1 and Mdm35 are colored in light brown and pink, respectively, and the Ω-loop of Ups1 in blue. The DLPA molecule is shown as a stick model with C yellow, O red, and P orange.

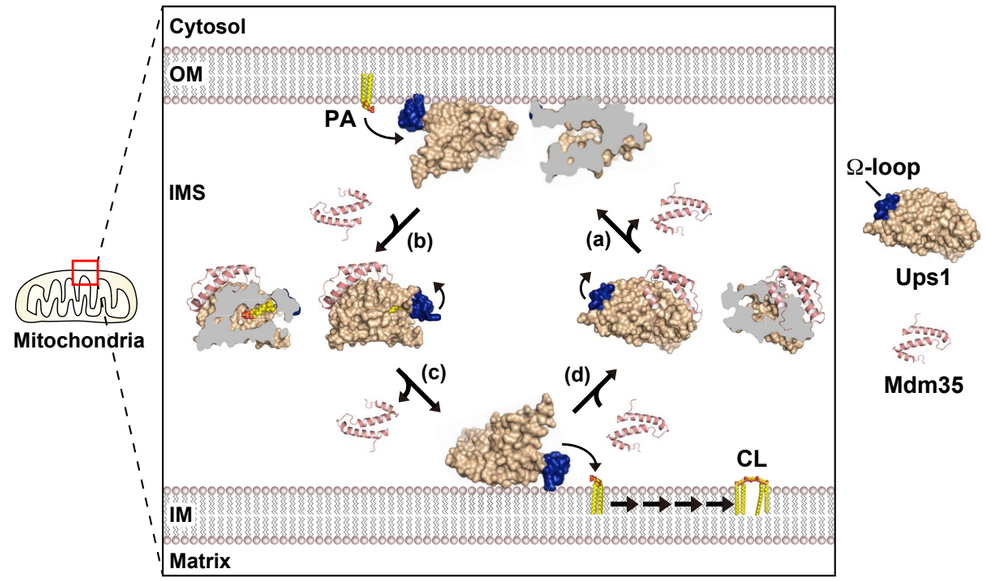

Figure 2: Model of the PA transfer between the mitochondrial OM and IM by the Ups1-Mdm35 complex. Ups1 and Mdm35 are shown by surface diagram and ribbon diagram, respectively, and PA and CL in space-filling form. The Ω-loop lid of Ups1 is indicated in dark blue.

Previously, Mdm35 was found to dissociate from Ups1 upon binding to the membrane containing acidic phospholipids [1], yet it is not clear if this Mdm35 dissociation is prerequisite for the Ups1 binding to the membrane for PA transfer. We thus constructed a covalently tethered Ups1-Mdm35 complex, in which Mdm35 dissociation from Ups1 is prevented. The liposome flotation assay and lipid transfer assay using these Ups1-Mdm35 tethering mutants demonstrated that dissociation of the Ups1-Mdm35 complex is important for stable binding of Ups1 to the membrane and its PA transport.

Taken together, we propose the following model of the PA transfer between the OM and IM by the Ups1-Mdm35 complex. Transient dissociation of the Ups1-Mdm35 complex allows Ups1 to bind to the OM (**Fig. 2a**), then opening of the Ω-loop lid promotes loading of PA from the OM into the pocket of Ups1. Closure of the pocket by the Ω-loop lid hides the hydrophobic PA from the aqueous environment and re-binding of Mdm35 to Ups1 allows Ups1 with loaded PA to leave the OM (**Fig. 2b**). The Ups1-Mdm35 complex with concealed PA in the pocket crosses the IMS, an aqueous divide between the OM and IM, to reach the IM. To release PA from the pocket of Ups1 into the IM, Mdm35 dissociates from Ups1 again to facilitate Ups1 binding to the IM rich in acidic CL, and the Ω-loop lid opens the pocket again (**Fig. 2c**). After PA is unloaded and released into the IM, Ups1 forms a complex with Mdm35 and leaves the IM (**Fig. 2d**). In conclusion, the crystal structures of the Ups1-Mdm35 complex with and without PA provide a mechanistic insight into the PA transfer between the mitochondrial OM and IM.

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BEAMLINES

AR-NE3A and AR-NW12A

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