Molecular architecture of lipid transfer between the two mitochondrial membranes

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Lipid biosynthesis occurs mostly in the endoplasmic reticulum (ER) and the distribution of lipid molecules between different organelle membranes is therefore vital for establishing membrane lipid composition and identity. The inner mitochondrial membrane requires tight control of its composition to maintain crucial functions, such as respiration. The mechanism of lipid transfer between the inner and outer mitochondrial membrane is largely unknown, although several proteins have been identified as mitochondrial lipid transfer proteins. The arrangement of these proteins between the inner and outer mitochondrial membrane, and how they carry out lipid transfer *in vivo*, are not known. We aim to understand lipid transfer between inner and outer membranes of mitochondria in *Saccharomyces cerevisiae*. We use an integrative approach to reveal the molecular mechanism of lipid transfer, and the arrangement of its protein components within the intermembrane space. In particular, cryo-electron tomography (cryo-ET) is used to obtain structural information about organization and architecture of lipid transfer components, and their influence on mitochondrial ultrastructure. We observe alterations in mitochondrial morphology when lipid transfer proteins are deleted, as well as more subtle changes, e.g. dilution of mitochondrial matrix, which can only be observed with cryo-ET. Elements of mitochondrial ultrastructure, such as intermembrane distances and membrane thickness, are also quantified in different deletion strains. Furthermore, we observe bridge-like connections between the inner and outer mitochondrial membrane. Quantifying the occurrence of these bridges shows that there is a decrease when lipid transfer proteins are deleted, indicating possible identity of these bridges. Subtomogram averaging will be used to further investigate the structure and possible composition of these bridges. In addition to cryo-ET, live fluorescence imaging is used to obtain insights into the specific roles of individual protein components involved in lipid transfer. Furthermore, our study addresses how lipid transfer events are distributed, relative to landmarks in mitochondrial structure such as cristae junctions and lipid transferring contact sites to the ER, and thus how lipid transfer connects to other mitochondrial functions.