

High resolution analysis of lipid droplet – membrane interactions in yeast

D. Kolb¹, H. Wolinski¹, R.I Koning², G. Leitinger³, J. Neumüller⁴, G. Zellnig⁵, A.J. Koster², S.D. Kohlwein¹

dagmar.kolb@uni-graz.at

1. Institute of Molecular Biosciences, Department of Biochemistry, University of Graz, Austria.
2. Leiden University Medical Center, Department of Molecular Cell Biology, The Netherlands.
3. Core Facility Ultrastructure Analysis, Center for Medical Research (ZMF), Medical University of Graz, Austria.
4. Center for Anatomy and Cell Biology, Department of Cell Biology and Ultrastructure Research, Medical University of Vienna, Austria.
5. Institute of Plant Science, University of Graz, Austria.

Keywords: TEM, electron tomography, lipid droplets, organelle membrane interaction, *Saccharomyces cerevisiae*

Lipid droplets (LD) form the main lipid storage organelles for neutral lipids in all eukaryotic cells. In recent years, LD have been extensively studied in different model organisms including the yeast *Saccharomyces cerevisiae*, due to their important role in cellular lipid and energy metabolism. LD are surrounded by a phospholipid monolayer harbouring a subset of proteins promoting the synthesis or mobilization of lipids. However, many molecular and biochemical details of neutral lipid metabolism are known, the biogenesis of these organelles and their interactions with other subcellular structures remain largely elusive.

Recent data obtained in our lab using four-dimensional live cell imaging suggest a close association of yeast LD with the endoplasmic reticulum (ER) membranes throughout the cell cycle [1]. To support these findings we have applied transmission electron microscopy serial-sectioning and dual axis electron tomography to study the cellular organization of lipid droplets in relation to the endoplasmic reticulum in wildtype and different mutant strains defective in lipid synthesis and mobilization. The tilt series was then used to calculate a tomogram of the acquired sample by weighted back-projection. Three-dimensional reconstructions of LD and ER were generated using EM 3D (Stanford University) and Amira™ (Mercury Computer Systems, Inc.) visualization packages, by surface rendering and thresholding [2].

We revealed that yeast lipid droplets in wild type but also in different analysed yeast mutant strains indeed show a close proximity to ER membranes, independently of their size and subcellular position. Based on these data a new model for the biogenesis and degradation of lipid droplets during various physiological states will be discussed.

1. Wolinski et al. (2009) unpublished.
2. Frank J. 2005. Electron tomography, Methods for Three-Dimensional Visualization of Structures in the Cell. Second edition, Springer.

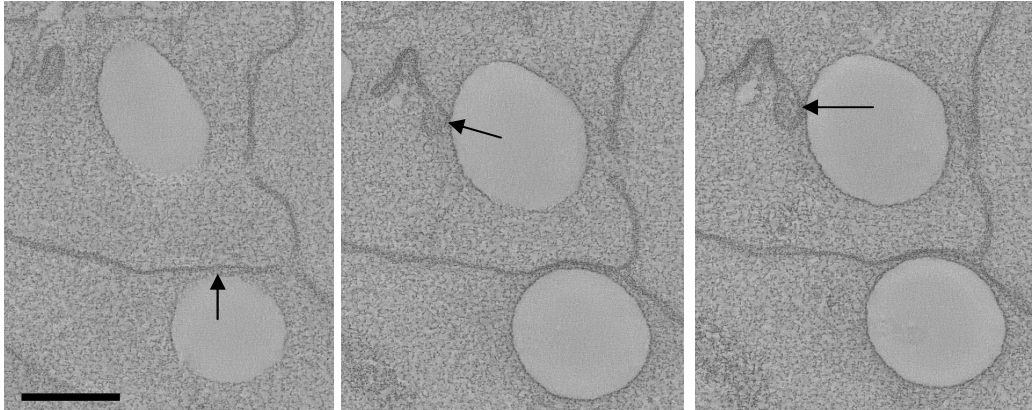


Figure 1. Snapshots of a series of electron tomography images showing lipid droplets in close proximity to endoplasmic reticulum membranes. Arrows indicate interaction between LD and ER. Bar=100nm.