LIPID DROPLET BIOGENESIS: NOVEL INSIGHTS FROM SUPER-RESOLUTION APPROACHES

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Lipid droplets (LD) are ubiquitous cellular storage compartments for neutral lipids that serve vital functions as an energy source. Multiple highly dynamic metabolic processes taking place on LD coordinate organismal lipid homeostasis but are also potential sources of lipid-associated disorders. Despite there crucial functions, precise origin and mechanisms of LD biogenesis are still controversial due to the lack of appropriate high-resolution techniques to investigate initial events of their formation.
Electron tomography of yeast mutant models of LD formation for the first time indicated an origin of neutral lipid deposition in the lumen of the endoplasmic reticulum. To confirm these observations and potentially obtain a better insight into the dynamics of this process we are developing and implementing STORM (STochastic Optical Reconstruction Microscopy) and PALM (PhotoActivated Localization Microscopy) approaches to unveil early events of LD formation. We are currently developing dual color labeling using PAINT (Point Accumulation In Nanoscale Topography) with the LD-specific lipophilic dye Nile Red and specifically tagged ER and LD-resident proteins to achieve sufficient temporal and spatial resolution to resolve the origin and dynamics of LD formation. In a static approach to unveil the architecture of the LD-membrane interface we further develop ‘expansion microscopy’, which may improve resolution up to three-fold while preserving structural interactions. Removal of the yeast cell wall appears to be crucial to avoid diffraction artifacts that hamper resolution, while stabilizing cellular content in a polymer matrix minimizes intracellular movement of flexible membranous structures even in chemically fixed cells.

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