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Authors
Li, Zhipeng
Olzmann, James A

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A Proteomic Map to Navigate Subcellular Reorganization in Fatty Liver Disease

Zhipeng Li[1] and **James A. Olzmann**[1,*]

[1]Department of Nutritional Sciences and Toxicology, University of California, Berkeley, Berkeley, CA 94720, USA

Abstract

In a tour de force in this issue of *Developmental Cell*, Krahmer et al. leverage proteomic platforms to reveal subcellular proteome dynamics during development of nonalcoholic fatty liver disease. Their findings uncover a remarkable degree of subcellular reorganization, alterations in protein localization, and remodeling of organelle contact sites.

Compartmentalization of biochemical reactions within specialized membrane-bound organelles is a defining characteristic of eukaryotic cells that provides many advantages. However, the need for coordinated cellular activities necessitates mechanisms for inter-organelle communication. Membrane contact sites formed between juxtaposed organelles provide sites for the regulated transfer of ions and lipids and act as principal regions for inter-organelle communication, (Toulmay and Prinz, 2011). The maintenance of cellular lipid homeostasis provides an exquisite example of coordinated inter-organelle activities, involving a beautifully orchestrated symphony of enzymatic reactions, metabolite-sensing mechanisms, and regulated cellular responses that occur at a myriad of subcellular locations —the plasma membrane, endoplasmic reticulum (ER), peroxisomes, mitochondria, and lipid droplets (LDs) (Toulmay and Prinz, 2011).

Dysregulation of lipid metabolism underlies prevalent metabolic diseases. For instance, ectopic deposition of triacylglycerol in LDs in the liver is a pathological hallmark of obesity-related hepatic steatosis, also known as nonalcoholic fatty liver disease (NAFLD). While fatty liver is often reversible, progression to nonalcoholic steatohepatitis (NASH) is associated with permanent liver damage and an increased risk for hepatocellular carcinoma. The formation of LDs during NAFLD is likely to be, at least initially, a protective mechanism to prevent lipotoxic damage arising from the free fatty acids and their flux into toxic lipid species. However, the long-term pathogenic impact of enlarged LDs as well as the molecular mechanisms underlying the progression of NAFLD to NASH are incompletely understood.

LDs are neutral lipid storage organelles that engage in nutrient-regulated interactions with nearly all organelles (Valm et al., 2017) and function as central hubs of lipid metabolism (Walther et al., 2017). LDs consist of a neutral lipid core encircled by a phospholipid

*Correspondence: olzmann@berkeley.edu.

monolayer decorated with integral and peripheral proteins that regulate LD functions, including triacylglycerol metabolism and the interaction of LDs with other organelles (Bersuker and Olzmann, 2017; Walther et al., 2017). However, the mechanisms that regulate the composition of the LD proteome and the association of LDs with other organelles remain mostly unknown. In addition, although recent advances in proteomic approaches, such as proximity labeling (Bersuker et al., 2018) and protein correlation profiling (Krahmer et al., 2013), enabled the identification of high-confidence LD proteomes in cultured cell lines, elucidating the dynamics of LD proteomes *in vivo* remains a mostly unmet challenge.

In a tour de force published in this issue of *Development Cell*, Krahmer et al. (2018) integrate protein correlation profiling methods and phosphoproteomics to provide a systems-level view of the changes in protein subcellular distribution and phosphorylation state that occur in mouse liver during the development of high-fat diet (HFD)-induced insulin resistance and NAFLD. Protein correlation profiling employs proteomic analysis of biochemically fractionated tissue homogenates to generate subcellular distribution profiles for each protein. In total, Krahmer et al. (2018) assigned subcellular localizations to ~6,000 proteins and over 16,000 phosphopeptides in livers isolated from mice fed a low-fat diet (LFD) or a HFD for 3 or 12 weeks. At 3 weeks HFD, at which point mice were insulin resistant but did not exhibit elevated triacylglycerol levels, relocalization in regulators of signaling pathways and transcription factors was observed (e.g., KRAS, RAF1, and GNAS). However, the most striking changes were found after 12 weeks HFD, following the development of NAFLD. In addition to the upregulation of lipid metabolism-related proteins, over 20% of the proteome exhibited altered localizations and a significant portion (~6%) redistributed into LD fractions. Protein association with LDs is impacted by protein crowding and competition for limited membrane surface (Kory et al., 2015). Thus, the exorbitantly large LDs in NAFLD may provide increased accessible surface area, permitting anomalous insertion of proteins into LDs and acting as a protein “sink” that depletes proteins (Figure 1), potentially causing impairments in a wide variety of cellular processes.

Several proteins implicated as tethers at membrane contact sites exhibited altered distributions in NAFLD. For example, the membrane contact site proteins VPS13A/D (ER-mitochondrion and ER-lipid droplet) and ESYT2 (ER-plasma membrane) showed a partial LD distribution following the development of NAFLD (Figure 1). This observation raises the intriguing possibility that increased formation of LD contacts with the plasma membrane, ER, and mitochondria facilitates the flux of absorbed lipids into LDs for storage during NAFLD. An increased association of LDs with mitochondria was confirmed by electron microscopy, potentially enabling transfer of fatty acids to mitochondria for β-oxidation or supporting ATP-dependent triacylglycerol synthesis and LD expansion. Alterations in the phosphorylation of VPS13A/D and ESYT2 correlated with their redistribution, but whether these phosphorylation events impact their localization and/or organelle tethering functions is unknown.

Perhaps the most remarkable alteration was in the distribution of Golgi proteins, which exhibited a near-complete redistribution into LD fractions following the development of NAFLD (Figure 1). Centrifugation was unable to separate the Golgi from LDs. Confocal and super-resolution microscopy confirmed that the Golgi apparatus was largely associated with

LDs in NAFLD, supporting a direct interaction between the Golgi and LDs. A similar redistribution into LD fractions was observed for COPI, which mediates retrograde vesicular transport in the secretory pathway and regulates LD monolayer surface tension (Wilfling et al., 2014). Examination of hepatocytes isolated from the HFD mice revealed a general reduction in protein secretion, including the secretion of apolipoprotein B100, an essential component of very low-density lipoproteins (VLDLs) (Figure 1). Starvation in serum-free medium led to LD degradation and corrected the secretory function, suggesting that the aberrant LDs, and perhaps their association with the Golgi or COPI, inhibit the secretory pathway. The reduction in VLDL secretion in response to HFD has been appreciated for decades, but the reason for this secretory impairment has remained a mystery. The strong association of the Golgi and COPI with LDs identified by Krahmer et al. (2018) provides a potential mechanism for this enigmatic trafficking defect. It is important to note that LD inhibition of VLDL secretion could induce a deleterious positive feedback loop in which lipid accumulation impairs VLDL secretion, leading to further intracellular lipid accumulation.

NAFLD is rapidly becoming the most prevalent liver disease, and treatment options beyond dietary interventions are lacking. The study from Krahmer et al. (2018) provides a spectacular resource (http://nafld-organellemap.org) that reveals global subcellular reorganization during NAFLD. Alterations in LD-organelle contacts could have large impacts on lipid metabolism, and understanding the composition of LD tethering complexes, their regulation, and their impact on lipid flux are important future directions. In addition, elucidating the mechanisms of Golgi association with LDs may provide insights into the pathogenesis of NAFLD and progression to NASH, potentially identifying therapeutically relevant targets to rescue VLDL secretion. This study demonstrates the power of quantitative proteomics to uncover significant disease-associated subcellular restructuring events *in vivo*, opening the door for similar proteomic studies of other disease models.

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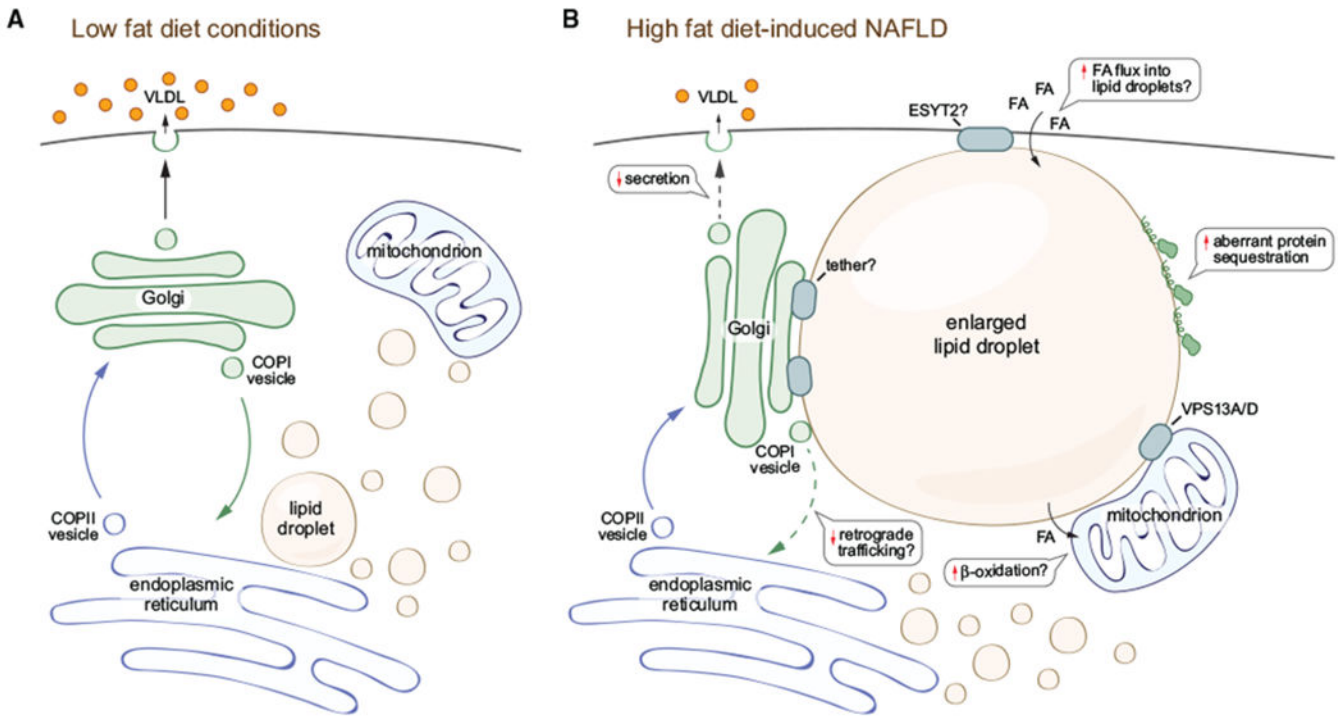

Figure 1. Subcellular Reorganization and Impaired Secretion in NAFLD

(A and B) The development of NAFLD is associated with a dramatic subcellular reorganization, including aberrant protein sequestration on LDs and increased interactions of LDs with the plasma membrane, mitochondria, and Golgi. FA, fatty acid. VLDL, very low-density lipoprotein.