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Endoplasmic Reticulum-Vacuole Contact Sites "Bloom" With Stress-Induced Lipid Droplets

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Abstract

Lipid droplets (LDs) serve as specialized cytoplasmic organelles that harbor energy-rich lipids for long-term storage and may be mobilized as nutrient sources during extended starvation. How cells coordinate LD biogenesis and utilization in response to fluctuations in nutrient availability remains poorly understood. Here, we discuss our recent work revealing how yeast spatially organize LD budding at organelle contacts formed between the endoplasmic reticulum and yeast vacuole/lysosome (sites known as nucleus-vacuole junctions [NVJs]). During times of imminent nutrient exhaustion, we observe blooms of stress-induced LDs surrounding the NVJ and find that this LD clustering is regulated by NVJ-resident protein Mdm1. We also discuss several emerging studies revealing specific proteins that demarcate a subpopulation of NVJ-associated LDs. Collectively, these studies reveal a previously unappreciated role for the spatial compartmentalization of LDs at organelle contacts and highlight an important role for interorganellar cross talk in LD dynamics under times of nutritional stress.

Keywords

nucleus-vacuole junction; lipid droplet; Mdm1; lipophagy; endoplasmic reticulum

Life is tough, and to survive, cells must deal with a barrage of stresses including fluctuations in nutrient availability. Upon sensing a decline in nutrients such as sugar, the budding yeast Saccharomyces cerevisiae responds by producing lipid droplets (LDs), a specialized organelle enriched with high-energy triacylglycerides (TAGs) that serves as an energy reservoir in a nutrient-poor environment. Starvation-induced LD biogenesis is highly conserved in metazoans, and recently, mammalian cells have been observed to increase their lipid stores when facing amino acid starvation (Nguyen et al., 2017). Ultimately, starvation-induced LDs will be harvested in catabolic metabolism by oxidative organelles like

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mitochondria and peroxisomes, thus allowing cells to subsist until better times arrive (Barbosa et al., 2015; Seo et al., 2017).

How do cells sense starvation and remodel their metabolism to cope with the harsh realities of subsistence living? Moreover, how do they coordinate the production of energy-rich LDs at a time when they are facing an energy crisis? In our recent article, we shed some light on these questions (Hariri et al., 2018). We demonstrated that, upon sensing a drop in nutrients, yeast spatially organizes starvation-induced LDs around the contact site formed between two organelles: the anabolic endoplasmic reticulum (ER) where LDs are made and the catabolic vacuole/lysosome where LDs will eventually be harvested (Hariri et al., 2018). This contact site—called the nucleus-vacuole junction (NVJ)—thus serves as a platform to conveniently cluster LDs at the physical interface between anabolic and catabolic metabolism (Figure 1(a) and (b)).

Initially discovered in 2000, the NVJ is familiar to any yeast biologist interested in interorganelle cross talk (Pan et al., 2000). Even during its original characterization, the NVJ was linked to lipid homeostasis, as numerous lipid-binding proteins such as Osh1 were found to be enriched there (Levine & Munro, 2001). More recently, studies have begun revealing a role for the NVJ in cellular metabolism. In addition to Osh1, other proteins involved in lipid metabolism including Nvj2 have been observed to dynamically relocalize from other regions of the cell to the NVJs of yeast cultured under specific growth conditions, suggesting the NVJ could harbor metabolic proteins in a context-dependent manner (Toulmay & Prinz, 2012). Using quantitative light microscopy and transmission electron microscopy imaging, our group screened many of these growth conditions and found that, in addition to harboring proteins, the NVJ physically expands when yeast are stressed. Indeed, NVJ expansion correlates with a transcriptional upregulation of *NVJ1* mRNA, and this is triggered by stresses including sugar depletion, amino acid starvation, and the addition of the TORC1-inhibitor rapamycin, indicating that the NVJ expands in response to nutrient deprivation in particular (Hariri et al., 2018).

So, the NVJ expands under stress conditions, but how does that relate to lipid metabolism? As stress response organelles, LDs are created by the ER in response to various stimuli. Although we know what enzymes are required to make LDs, very little is known about what defines where LDs bud within the vast ER network. Using time-lapse imaging, we demonstrated that the NVJ indeed serves as a site for LD budding. This was achieved by clearing yeast of their preexisting LDs through treatment with the drug cerulenin, then washing out the drug, and imaging single cells over time. Strikingly, nascent LDs were observed to *bloom* at the NVJ, confirming it as a site for LD biogenesis (Hariri et al., 2018). However, how the NVJ becomes a *hotspot* for LD biogenesis under stress was unclear.

Part of the answer came by examining the protein tethers that create the NVJ. At least two proteins are required for NVJ formation: the tethering protein Nvj1 and a less understood protein called Mdm1 that we recently showed to be sufficient to connect the ER and vacuole in *nvj1*-deficient yeast (Henne et al., 2015). Equipped with this knowledge, we used high-resolution three-dimensional-structured illumination microscopy (SIM) imaging to demonstrate that these two proteins can be spatially distinguished at the NVJ. Whereas Nvj1

is evenly distributed across the entire NVJ contact, Mdm1 forms distinct foci only at the NVJ periphery, exactly where LDs accumulate under nutritional stress.

This suggested that Mdm1 may play a role in NVJ-associated LD budding. Indeed, two experiments indicate that this is the case. In one, we overexpressed Mdm1, a condition previously shown to drive NVJ expansion. However, we conducted this experiment with yeast growing in nutrient-rich media, a condition when LDs do not normally cluster at the NVJ. Strikingly, Mdm1 overexpression is sufficient to drive LD accumulation at the NVJ even in nonstressed cells, indicating that modulating Mdm1 expression somehow mimics the stress-induced LD accumulation at the NVJ. In a second experiment, we generated yeast deleted for both *nvj1* and *mdm1*—therefore with no NVJ—and then reintroduced Mdm1 alone into this mutant. Upon feeding the yeast oleate to stimulate LD biogenesis, we observe LDs budding at Mdm1-positive ER-vacuole contact sites. Here, Mdm1 appears to *cup* the growing LDs adjacent to the vacuole, suggesting that Mdm1 directly interacts with LDs and is sufficient to localize them to ER-vacuole junctions (Henne et al., 2015). We also examined whether loss of the NVJ itself could affect LD production, finding that NVJ-deficient yeast exhibit defects in LD biogenesis and maturation.

How does Mdm1 contribute to LD budding at the NVJ? To address this question, we immunoprecipitated Mdm1 and conducted mass-spectrometry-based proteomics to identify its binding partners. Intriguingly, this revealed fatty acyl-CoA synthases including Faa1, as well as Fas1 and Fas2, components of the highly conserved fatty-acyl synthase complex (Hariri et al., 2018). These proteins are responsible for activating free fatty acids through conjugation with CoA, a step essential to their entry into any lipid metabolic pathway. Consistent with the coprecipitation, we observed Faa1-GFP decorating the surface of NVJ-associated LDs in yeast undergoing diauxic shift, suggesting these LDs were growing by actively incorporating fatty acids, and also that Mdm1 may associate with LDs by binding Faa1. This observation is similar to previous work from the Siniossoglou group demonstrating that Pah1, the enzyme that generates diacylglyeride, also targets to the NVJ during diauxic shift (Barbosa et al., 2015). Indeed, since diacylglyeride requires an additional fatty acyl-CoA to form TAG at LDs, an intriguing model is that proteins including Mdm1, Pah1, and Faa1 may together coordinate the synthesis of TAG at NVJ-associated LDs (Figure 1(c)).

Thus, the NVJ appears to spatially coordinate starvation-induced LD biogenesis in yeast, providing a convenient locale for both the lipids and enzymes required for making droplets. An explanation for accumulating LDs near the vacuole could be that this LD placement is strategic for cell survival, since many LDs will eventually be transferred to the vacuole and digested via lipophagy (Wang et al., 2014). Creating or clustering them in a subregion of the ER network adjacent to the vacuole may therefore make for easy transfer to the vacuole surface and eventual vacuolar digestion. A second and equally intriguing idea is that clustering LDs in specific cellular subregions may allow them to deliver specific lipids to other organelles like the vacuole.

Are there specific proteins that demarcate this NVJ-associated LD subpopulation? Very likely, as two new studies from Maria Bohnert’s and Pedro Carvalho’s groups independently

identify two new LD organization proteins—Ldo16 and Ldo45—which like Mdm1 regulate LD positioning at the NVJ (Eisenberg-Bord et al., 2018; Teixeira et al., 2018). Their work also suggests that LD organization proteins may be necessary to move preexisting LDs to the NVJ, implying that mechanisms exist to both create and attract LDs to this contact site. Future studies will no doubt continue to reveal additional roles for spatially compartmentalizing LDs. It will also be interesting to see whether this high degree of spatial compartmentalization is a conserved strategy in mammals.

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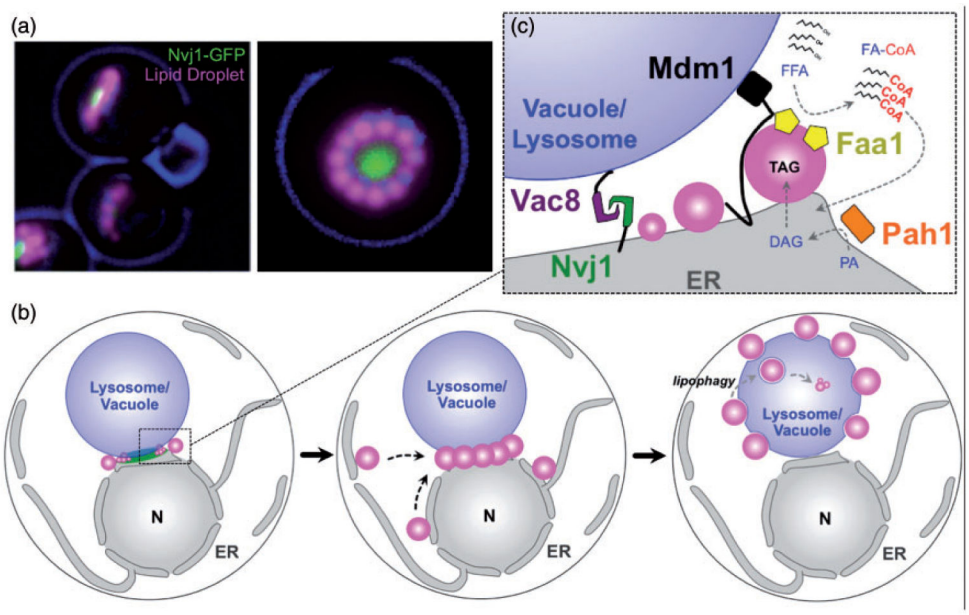

Figure 1.

Yeast NVJs accumulate nutrient stress-induced LDs. (a) Nvj1-GFP (green) expressing yeast stained with lipid droplet marker AutoDOT (magenta) display a ring-like accumulation of LDs when imaged during diauxic shift growth. (b) Time-course of LD accumulation at the NVJ showing initial LD budding at the NVJ periphery (left), followed by the formation of LD pearl necklaces that surround the NVJ (center), and finally their transfer to the vacuole surface for lipophagy (right). (c) Cartoon model of yeast budding LDs at the NVJ. Mdm1 (black) associates with NVJ-associated LDs and interacts with PI3P on the vacuole via a PX domain (square), the ER via a transmembrane domain, and with fatty acyl-CoA synthase Faa1 (yellow), which converts free fatty acids to fatty acyl-CoA. Pah1 (orange) forms DAG at the NVJ prior to its conversion to TAG. Note that these LDs are also positive for Ldo45/16 (not shown). Scale bar: 1 μm. FFA=free fatty acids; FA-CoA=fatty acyl-CoA; ER=endoplasmic reticulum; DAG=diacylglyeride; TAG=triacylglyeride; PA= phosphatidic acid.