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The Vacuole Lipid Droplet Contact Site vCLIP

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Abstract

Lipid droplets frequently form contact sites with the membrane of the vacuole, the lysosome-like organelle in yeast. These vacuole lipid droplet (vCLIP) contact sites respond strongly to metabolic cues: while only a subset of lipid droplets is bound to the vacuole when nutrients are abundant, other metabolic states induce stronger contact site formation. Physical lipid droplet-vacuole binding is related to the process of lipophagy, a lipid droplet-specific form of microautophagy. The molecular basis for the formation and function of vCLIP contact sites remained enigmatic for a long time. This knowledge gap was filled when it was found that vCLIP is formed by the structurally related lipid droplet tether proteins Ldo16 and Ldo45, and the vacuolar surface protein Vac8. Ldo45 additionally recruits the phosphatidylinositol transfer protein Pdr16 to vCLIP. Here, we review the literature on the lipid droplet-vacuole contact site in light of the progress in our understanding of its molecular basis and discuss future directions for the field.

Keywords

Ldo16, Ldo45, Pdr16, Vac8, LDAF1, seipin, lipophagy, vCLIP, contact site, lipid droplet, vacuole, lysosome

Introduction

Lipid droplets are the cell's dedicated lipid storage organelles. They consist of a central compartment composed of neutral lipids, such as triglycerides and sterol esters, and an outer phospholipid monolayer that houses the lipid droplet proteome. Lipid droplets are formed at the membrane of the endoplasmic reticulum (ER) (Thiam et al., 2013; Nettebrock and Bohnert, 2020; Klemm and Carvalho, 2024). Neutral lipids synthesized by ER resident enzymes form lens-shaped structures between the leaflets of the ER membrane, which grow and finally bud toward the cytosol. Mature lipid droplets frequently maintain a tight link to the ER via contact sites (Olzmann and Carvalho, 2019; Salo and Ikonen, 2019; Hugenroth and Bohnert, 2020). Contact sites are areas of close proximity between organelles that mediate collaborative functions, by enabling interorganelle exchange of material and information, by locally enriching functional proteins, and by determining organelle positioning and segregation (Scorrano et al., 2019). Structurally, contact sites depend on specialized tether proteins that physically attach the membranes of distinct organelles to each other (Eisenberg-Bord et al., 2016; Bohnert, 2020a). ER-lipid droplet contact sites additionally contain lipidic stalk structures that directly link the outer leaflet of the ER membrane to the phospholipid monolayer of the lipid droplet (Jacquier et al., 2011; Kassan et al., 2013). A key player in lipid droplet biogenesis from the ER and in maintenance of fully functional lipid droplet-ER contact sites is the seipin machinery (Salo, 2023). Seipin forms oligomeric complexes in the ER membrane and at ER-lipid droplet interfaces that spatially organize lipid droplet formation and lipid droplet-ER communication (Walther et al., 2023). Seipin dysfunction results in the human disease Berardinelli-Seip congenital lipodystrophy (Magré et al., 2001). While the spatial and functional interplay of lipid droplets with the ER has received particular attention in the past, we are starting to understand that lipid droplets engage extensively in contact sites with a range of different partner organelles (Valm et al., 2017; Shai et al., 2018). Alterations of lipid droplet-organelle contact sites are linked to metabolic and infectious disease (Herker et al., 2021).

In *Saccharomyces cerevisiae* (from here on: yeast), a prominent lipid droplet contact site is the vCLIP, an interface with the lysosome-like vacuole. The name vaCuole LIPid droplet contact site or "vCLIP" was coined in 2018 in a systematic study that aimed at mapping the full extent of organelle contact sites in yeast (Shai et al., 2018). For simplicity,

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we will use the term vCLIP uniformly throughout this review when describing reports on the spatial coupling between lipid droplets and vacuoles, including those from prior to its naming. While vacuole lipid droplet contact sites have been frequently observed by microscopy, their molecular basis has long been enigmatic (Schuldiner and Bohnert, 2017). In 2024, two studies have filled this knowledge gap and identified the proteins that tether lipid droplets to the vacuolar membrane (Álvarez-Guerra et al., 2024; Diep et al., 2024). On lipid droplets, two structurally related proteins termed lipid droplet organization proteins of 16 and 45 kDa (Ldo16 and Ldo45) mediate formation of vCLIP contact sites. Tethering is achieved by an interaction of either Ldo protein with the vacuolar surface protein Vac8. A fourth vCLIP component is Pdr16, a phosphatidylinositol transfer protein that is recruited by Ldo45 (Figure 1).

Here, we will review the literature on lipid droplet-vacuole cooperation in light of the identification of the vCLIP protein machinery.

The Many Faces of Vacuole Lipid Droplet Contact Sites Through Metabolic States and Stress Conditions

Sites of close lipid droplet-vacuole apposition have been observed in multiple studies prior to the identification of the molecular vCLIP machinery. The contact site has raised interest particularly due to its conspicuous responsiveness to environmental cues such as nutrient availability and stress (Schuldiner and Bohnert, 2017) (Figure 1).

In exponentially growing cells that have access to ample nutrients, the majority of the cellular lipid droplet pool is not in direct contact with the vacuole. However, even at this state, sensitive bimolecular fluorescence complementation-based assays detect a small fraction of vCLIP-engaged lipid droplets (Shai et al., 2018; Diep et al., 2024). Typically, a subpopulation of just one or two lipid droplets per cell is vCLIP-positive at nutrient repletion, while the others are devoid of a vCLIP (Diep et al., 2024).

vCLIP abundance typically increases when cells experience less favorable conditions. Nutrient starvation regimes that promote vCLIP formation include both gradual and acute glucose restriction, phosphate restriction, and acute nitrogen depletion (Barbosa et al., 2015; Eisenberg-Bord et al., 2018; Hariri et al., 2018; Rogers et al., 2022; Álvarez-Guerra et al., 2024; Diep et al., 2024).

When cells gradually run out of glucose and have to shift their energy metabolism from glycolysis to respiration (a state termed diauxic shift), the spatial distribution of lipid droplets within the cell is altered. Lipid droplets accumulate in a special niche of the cell, adjacent to the nucleus vacuole junction NVJ, a contact site between the nuclear ER and the vacuole (Wang et al., 2014; Eisenberg-Bord et al., 2018; Hariri et al., 2018; Ferreira and Carvalho, 2021). A similar effect on lipid droplet localization can be induced by acute experimental glucose restriction (Rogers et al., 2022; Diep et al., 2024). Acute glucose restriction also induces alterations in lipid droplet composition and structure. Pronounced lipolysis mediates a decrease in the triglyceride-to-sterol ester ratio, formation of liquid crystalline sterol ester latices within the neutral lipid core, and alterations in the lipid droplet surface proteome (Rogers et al., 2022). How these changes relate to lipid droplet localization at the NVJ is so far unclear. The main NVJ tether consists of the nuclear ER protein Nvj1 and the vacuolar surface protein Vac8 (Pan et al., 2000) (Figure 1). Additionally, the NVJ houses numerous lipid handling proteins, including the lipid transfer proteins Osh1, Lam6, and Nvj2 (Levine and Munro, 2001; Kvam and Goldfarb, 2004; Toulmay and Prinz, 2012; Elbaz-Alon et al., 2015; Gatta et al., 2015; Murley et al., 2015), and the lipid metabolism enzymes Tsc13, Hmg1, Hmg2, Cvm1, Pah1, and Faa1 (Kohlwein et al., 2001; Barbosa et al., 2015; Hariri et al., 2018; Rogers et al., 2021; Bisinski et al., 2022). The overall size of the NVJ increases upon glucose deprivation (Hariri et al., 2018), an effect that is dependent on the NVJ regulatory component Snd3 (Tosal-Castano et al., 2021). While some NVJ proteins reside at the contact site constitutively, others are recruited only in response to nutrient restriction (Bohnert, 2020a). Pex30 and Pex29, two structurally related ER membrane proteins, localize to the NVJ during glucose exhaustion. Loss of these proteins results in a structurally compromised NVJ, and also affects lipid droplet clustering in this region (Ferreira and Carvalho, 2021). A further molecular player in cellular lipid droplet distribution is the NVJ tether protein Mdm1, which demarcates sites of lipid droplet formation close to the NVJ during the diauxic shift (Hariri et al., 2018) (Figure 1).

When glucose scarcity persists long-term, cells exit the cell cycle and enter a stationary phase. At this condition, lipid droplet accumulation at the NVJ decreases. Instead, a phenotype prevails at which lipid droplets appear to encircle the entire vacuole (Wang et al., 2014; Barbosa et al., 2015). This extensive vCLIP formation coincides with a segregation of the vacuolar membrane into liquid ordered and liquid disordered microdomains (Toulmay and Prinz, 2013; Wang et al., 2014). Later in stationary phase, after prolonged starvation, lipid droplets enter the vacuolar lumen in a microautophagy process termed lipophagy (Wang et al., 2014) (Figure 1). A reciprocal relationship between lipid droplets and vacuole membrane reorganization has been proposed, in which lipid droplets supply sterol for vacuole microdomain formation, while liquid ordered domains serve as docking sites for lipid droplets (Wang et al., 2014). Lipid droplet internalization into the vacuole during stationary phase furthermore depends on a range of proteins including core autophagy components (Wang et al., 2014), the Niemann-Pick type C proteins Ncr1 and Npc2 (Tsuji et al., 2017), and the phosphatidylinositol 4-kinases Stt4 and Pik1 (Kurokawa et al., 2020). In addition to stationary phase, a

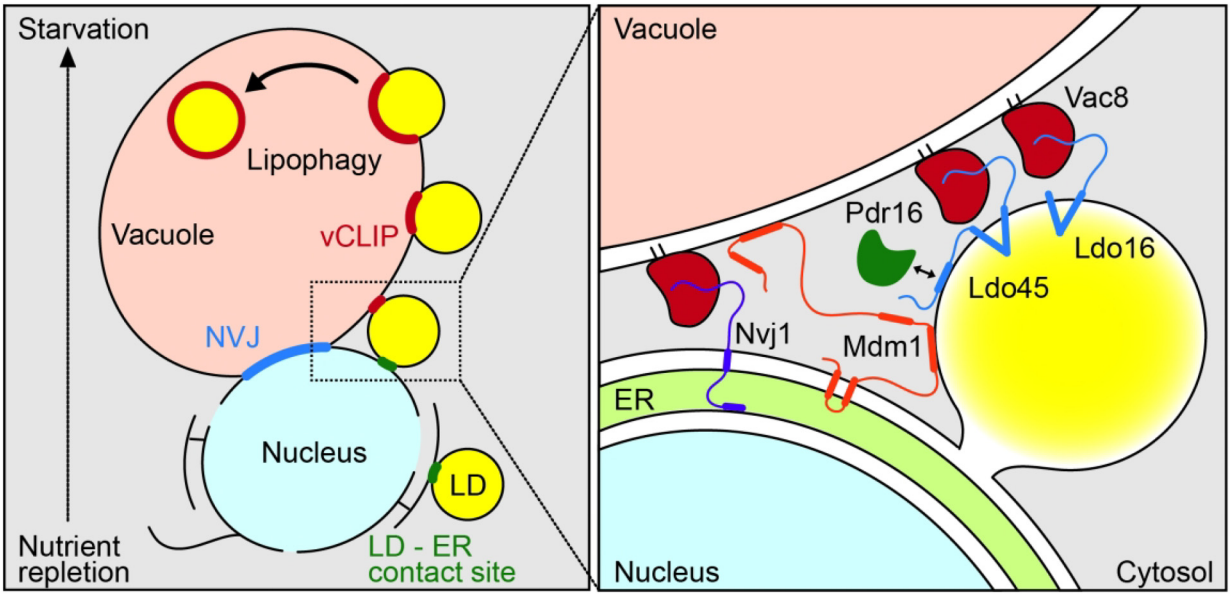

Figure 1. The vacuole lipid droplet contact site machinery vCLIP. (Left) Schematic representation of lipid droplet (LD) contacts with endoplasmic reticulum (ER) and vacuole membranes depending on metabolic states. At nutrient repletion, most lipid droplets are not bound to the vacuole. Early in glucose exhaustion, lipid droplets accumulate at the nucleus-vacuole junction NVJ by forming vacuole lipid droplet (vCLIP) contact sites. vCLIPs expand when starvation conditions persist, eventually leading to lipid droplet internalization into the vacuole by lipophagy. (Right) Schematic representation of the proteins involved in lipid droplet accumulation at the NVJ. Nvj1- Vac8 and Ldo16/45-Vac8 form the physical basis of the NVJ and the vCLIP, respectively. The two Ldo proteins are structurally related and share the same Vac8 binding domain. Ldo45 additionally comprises an N-terminal extension which recruits the phosphatidylinositol transfer protein Pdr16. The NVJ tether protein Mdm1 resides in the periphery of the NVJ, where it demarcates sites of lipid droplet formation.

range of further stimuli induce lipophagy, for example acute nitrogen starvation (van Zutphen et al., 2014; Tsuji et al., 2017; Kurokawa et al., 2020; Zhang et al., 2020), acute glucose reduction (Seo et al., 2017; Zhang et al., 2020), the diauxic shift (Oku et al., 2017), and different forms of ER stress including alterations in phospholipid synthesis and exposure to tunicamycin or dithiothreitol (Vevea et al., 2015; Garcia et al., 2021; Liao et al., 2021). The molecular determinants of the lipophagy process and the timescales of lipid droplet internalization differ dependent on the specific stress condition (Fairman and Ouimet, 2022). For example, the Endosomal Sorting Complexes Required for Transport (ESCRT) machinery differentially affects lipophagy dependent on the type of induction (Vevea et al., 2015; Oku et al., 2017; Kurokawa et al., 2020; Zhang et al., 2020; Garcia et al., 2021; Liao et al., 2021). Of note, vCLIP formation is not always linked to lipophagy, for example, extensive vCLIPs form upon phosphate starvation (Álvarez-Guerra et al., 2024), a condition that has been reported to result in massive lipid droplet accumulation in the cytosol and in a block in lipophagy (Peselj et al., 2022).

The Molecular Machinery of vCLIP Contact Sites

In 2024, two independent publications have described a key role of the Lipid Droplet Organization proteins Ldo16 and Ldo45 in vCLIP formation (Álvarez-Guerra et al., 2024; Diep et al., 2024) (Figures 1 and 2). Both Ldo proteins were originally described in 2018 as lipid droplet proteins that are structurally and functionally linked to the lipid droplet biogenesis factor seipin (Eisenberg-Bord et al., 2018; Teixeira et al., 2018; Bohnert, 2020b). The two Ldo proteins are derived from overlapping genes, so that the amino acid sequence of Ldo16 is identical to the most C-terminal part of Ldo45 (Miura et al., 2006; Eisenberg-Bord et al., 2018; Teixeira et al., 2018). Affinity purifications (Eisenberg-Bord et al., 2018; Teixeira et al., 2018), proximity ligation, and yeast two hybrid assays (Wang et al., 2024) show that Ldo16 and Ldo45 form a complex with the seipin subunits Sei1 and Ldb16. Consistent with a role of the Ldo proteins in lipid droplet biogenesis, alterations in Ldo expression affect lipid droplet morphology as well as the lipid droplet surface proteome (Eisenberg-Bord et al., 2018; Teixeira et al., 2018). Putative metazoan Ldo homologs are the human LDAF1/promethin, which also collaborates with seipin (Castro et al., 2019; Chung et al., 2019), and the Drosophila melanogaster protein dmLDAF1, likewise a seipin partner that affects fat storage in vivo (Chartschenko et al., 2021). Collectively, these findings indicate that Ldo/LDAF1 proteins have a role in lipid droplet formation together with seipin.

However, already during the original description of the links between the Ldo proteins and seipin, a number of

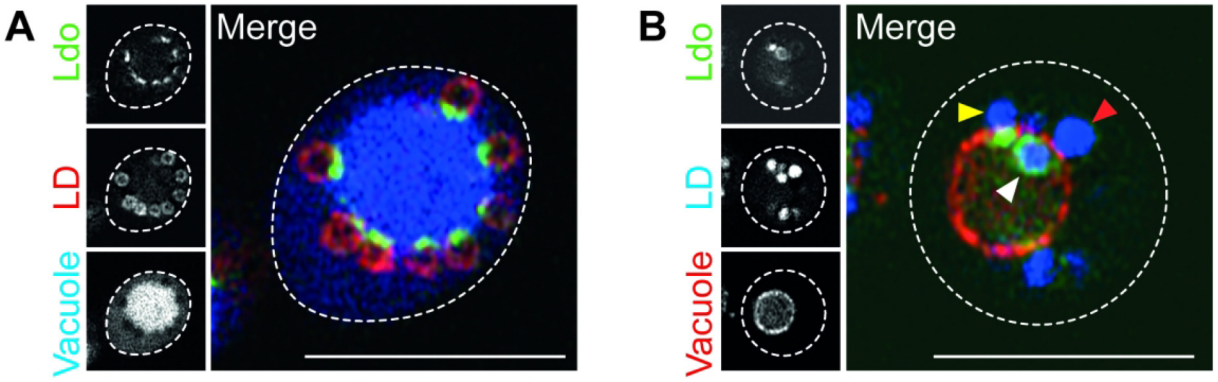

Figure 2. The Lipid Droplet Organization proteins localize to sites of tight vacuole-lipid droplet contact. (A) Cells expressing Ldo-GFP and the lipid droplet (LD) marker Erg6-mCherry were cultured overnight in synthetic medium with 2% glucose, and then transferred to glucose-free medium containing 0.2% oleic acid for 24 hours. Cells were stained with the vacuole lumen dye CMAC (7-amino-4-chloromethylcoumarin) and imaged on a ZEISS LSM980 Airyscan microscope. The Ldo proteins act as lipid droplet-vacuole tethers and are visible as defined foci at vacuole lipid droplet (vCLIP) contact sites. Scale bar, 5 µm. (B) Cells expressing Ldo-GFP and the vacuole membrane marker Vph1-mCherry were treated and analyzed as described in (A). Lipid droplets were visualized using the neutral lipid dye MDH (monodansylpentane). An untethered lipid droplet (red arrowhead), a vCLIP-engaged lipid droplet (yellow arrowhead), and a lipid droplet internalized into the vacuole (white arrowhead) are visible. Scale bar, 5 µm.

phenotypes were noted that appeared unrelated to the process of lipid droplet biogenesis. (i) At nutrient replete conditions, the Ldo proteins show a peculiar cellular distribution. Instead of being uniformly distributed across the cellular lipid droplet pool, they are strongly enriched on a subpopulation of lipid droplets that are positioned directly adjacent to the NVJ. These special lipid droplets are additionally enriched in further proteins, Pdr16, Tld1/Bsc2, Erg2, Tgl4, and Srt1 (Eisenberg-Bord et al., 2018). (ii) Loss of the Ldo proteins blocks the accumulation of lipid droplets adjacent to the NVJ that is typically observed during the diauxic shift (Eisenberg-Bord et al., 2018). (iii) Additionally, *ldo* mutants have a defect in the formation of vacuolar microdomains in stationary growth phase, as well as (iv) a defect in stationary phase lipophagy (Teixeira et al., 2018). A common characteristic of these different phenotypes is that they are related to the spatial orientation of lipid droplets toward the vacuole.

The mechanistic basis of these phenotypes was resolved early in 2024, when Diep et al. and Álvarez-Guerra et al. discovered that both Ldo proteins act as molecular tethers that mediate formation of the vCLIP contact site. Both Ldo16 and Ldo45 bind to lipid droplets via a hydrophobic domain. The tethering function of the proteins depends on an intrinsically disordered region C-terminal to this lipid droplet binding domain. vCLIP formation does not require the presence of seipin (Álvarez-Guerra et al., 2024; Diep et al., 2024), suggesting that Ldo16/45 are multifunctional proteins. The vacuolar Ldo binding partner for vCLIP formation is Vac8, a multifunctional armadillo repeat domain-protein (Álvarez-Guerra et al., 2024; Diep et al., 2024) (Figure 1). Besides its function in vCLIP, Vac8 has key roles at the NVJ, by acting as tether together with Nvj1 (Pan et al., 2000) and through its role in recruiting Lam6 to the NVJ (Elbaz-Alon et al., 2015; Murley et al., 2015). Additionally, Vac8 is the receptor for the myosin adapter protein Vac17, and thus mediates vacuole inheritance (Wang et al., 1996; Pan and Goldfarb, 1998; Wang et al., 1998; Ishikawa et al., 2003; Tang et al., 2003). Vac8 also has a role in vacuole fusion (Pan and Goldfarb, 1998; Veit et al., 2001). Furthermore, Vac8 is involved in bulk autophagy (Hollenstein et al., 2019; Gatica et al., 2021), and in several types of selective autophagy (Wang et al., 1996, 1998; Oku et al., 2006; Kissová et al., 2007; van Zutphen et al., 2014; Boutouja et al., 2019). While both Vac8 and Ldo are multifunctional, multiple lines of evidence indicate a direct role of Ldo-Vac8 complexes as vCLIP tethers. Overexpression of the proteins promotes vCLIP formation, while their loss abolishes tethering (Álvarez-Guerra et al., 2024; Diep et al., 2024). The Ldo proteins form defined foci at lipid droplet-vacuole interfaces (Figure 2) in a manner dependent on Vac8 (Álvarez-Guerra et al., 2024; Diep et al., 2024). Vac8 was also detected at vCLIPs using immuno electron microscopy (Álvarez-Guerra et al., 2024). Deletion of the Ldo lipid droplet binding domain yields a cytosolic Ldo variant that is recruited to the vacuolar membrane in a manner dependent on Vac8 (Diep et al., 2024). Synthetic targeting of the C-terminal intrinsically disordered Ldo domain to the surfaces of peroxisomes results in formation of peroxisome-vacuole contact sites (Diep et al., 2024), while synthetic targeting of Vac8 to the nuclear envelope recruits lipid droplets to the nucleus (Álvarez-Guerra et al., 2024). Vac8 can be co-purified with Ldo16 in affinity purifications from yeast cells (Diep et al., 2024), and direct Ldo-Vac8 binding was demonstrated upon heterologous protein expression (Álvarez-Guerra et al., 2024). Structure

predictions suggest that Ldo occupies the same Vac8 region like its partner protein Nvj1, and indeed, a mutual regulation of vCLIPs and NVJs was observed (Álvarez-Guerra et al., 2024). Together, these findings demonstrate that Ldo and Vac8 together form a vCLIP tether (Figure 1).

The molecular composition and the overall abundance of vCLIPs respond strongly to the metabolic state of the cell. At nutrient replete conditions, vCLIPs are restricted to a defined subpopulation of lipid droplets (Eisenberg-Bord et al., 2018; Diep et al., 2024). In contrast, vCLIP expands to the majority of lipid droplets in response to glucose starvation (Diep et al., 2024). Stationary phase lipophagy depends on the ability of cells to form vCLIPs (Álvarez-Guerra et al., 2024; Diep et al., 2024). The Vac8 binding domain of Ldo comprises a serine (position 102 in Ldo16/366 in Ldo45) that is phosphorylated by the cyclin dependent kinases Cdc28 and Pho85. The site becomes progressively dephosphorylated when cells approach conditions at which stationary phase lipophagy occurs, and phosphomimetic, but not non-phosphorylatable Ldo variants, induce a lipophagy defect (Diep et al., 2024). This suggests that the function of vCLIP in the lipophagy process is regulated via the Ldo phosphorylation state. Ldo16 and Ldo45 contain the same C-terminal tethering domain, and in principle, either Ldo protein can mediate vCLIP formation and lipophagy on its own (Álvarez-Guerra et al., 2024; Diep et al., 2024). However, expression levels of the two proteins change differentially in response to nutrient availability, with Ldo45 being more abundant at nutrient replete conditions, and Ldo16 becoming dominant for example in stationary phase (Teixeira et al., 2018; Álvarez-Guerra et al., 2024; Diep et al., 2024).

This is particularly interesting, because Ldo45, but not Ldo16, has been found to mediate targeting of the phosphatidylinositol transfer protein Pdr16 to lipid droplets (Eisenberg-Bord et al., 2018; Teixeira et al., 2018). Like the Ldo proteins, Pdr16 forms foci at lipid droplet-vacuole interfaces (Álvarez-Guerra et al., 2024; Diep et al., 2024), showing that this protein is a further vCLIP component (Figure 1). Pdr16 binds to an amphipathic helix in the N-terminal region of Ldo45 that is not present in Ldo16. vCLIP recruitment of Pdr16 depends on the physical coupling of the tethering function and the Pdr16 binding function of Ldo45 within the same protein (Diep et al., 2024). Pdr16 itself is not required for vCLIP formation, nor for vacuole microdomains or lipophagy (Álvarez-Guerra et al., 2024; Diep et al., 2024). In vitro data suggests a potential role of Pdr16 in intermembrane transfer of phosphatidylinositol and sterols (Li et al., 2000; Tripathi et al., 2019; Šťastný et al., 2023), and Pdr16 could thus act as vCLIP lipid transfer protein. Alternatively, Pdr16 may be involved in the local regulation of phosphoinositide signaling (Schaaf et al., 2008). Indeed, phosphoinositides have a role at vCLIPs that is not fully understood to date. Phosphatidylinositol-4-phosphate has been detected in the cytoplasmic leaflet of lipophagic vesicles, and inhibition of the phosphatidylinositol-4-kinases Stt4 and Pik4 affects lipophagy (Kurokawa et al., 2020). On the other hand, inactivation of the phosphoinositide phosphatase Sac1 promotes lipid droplet binding to the vacuolar membrane (Foti et al., 2001). Uncovering the exact role of Pdr16 at vacuole-lipid droplet interfaces is an important topic for the future. The presence of this protein at vCLIPs indicates that further vCLIP functions in and beyond lipophagy might await discovery.

Perspectives

Contact sites between lipid droplets and lysosome-like organelles are not restricted to yeast. In mammals, lipid droplet autophagy is mediated either by macro-autophagy (Singh et al., 2009) or by micro-autophagy (Schulze et al., 2020), with the latter process involving a direct lipid droplet-lysosome interplay. Lipid droplet-lysosome contacts have been observed in different mammalian cell types (Valm et al., 2017; Schulze et al., 2020; Menon et al., 2023; Miner et al., 2024). Similar to vCLIPs, formation of lipid droplet-lysosome contact sites is promoted by starvation and exposure to oleic acid in COS-7 cells (Valm et al., 2017). The small GTPases Rab7 (Schroeder et al., 2015), Rab10 (Li et al., 2016), and ARL8B (Menon et al., 2023) affect lipid droplet-lysosome interplay, but the molecular basis for organelle tethering is still incompletely understood, precluding detailed insights into the roles of the contact in lipid homeostasis.

Identification of the yeast vCLIP protein machinery opens the door to an in-depth functional analysis of the intriguing lipid droplet-vacuole interplay across metabolic stages. We know that during stationary phase, vCLIP is required for the process of lipophagy. Particularly in light of the regulation of lipid droplet internalization into the vacuole via the Ldo phosphorylation state (Diep et al., 2024), it will be exciting to uncover how the vCLIP machinery cooperates with the other proteins required for lipophagy (Wang et al., 2014; van Zutphen et al., 2014; Vevea et al., 2015; Oku et al., 2017; Seo et al., 2017; Tsuji et al., 2017; Teixeira et al., 2018; Kurokawa et al., 2020; Zhang et al., 2020; Garcia et al., 2021; Liao et al., 2021). More broadly however, multiple observations indicate that additional vCLIP functions besides lipophagy might exist. vCLIP has been detected at all conditions tested so far. This includes the observation of special vCLIP-engaged lipid droplet subpopulations at nutrient replete conditions (Shai et al., 2018; Diep et al., 2024), and extensive vCLIP formation during phosphate starvation (Álvarez-Guerra et al., 2024), a condition at which lipophagy appears blocked (Peselj et al., 2022). While not formally excluded, it seems unintuitive that these ubiquitous contact sites should solely serve the function of preparing for an eventual future induction of lipophagy. Furthermore, initial findings show that the molecular composition of the vCLIP machinery responds to metabolic cues. The vCLIP

components Ldo45 and Pdr16 are enriched at vCLIPs during nutrient repletion, but are less abundant in stationary phase (Álvarez-Guerra et al., 2024; Diep et al., 2024). This suggests that vCLIP-engaged lipid droplets at glucose repletion might serve unique roles beyond lipophagy. In support of this, Ldo45, but not Ldo16, counteracts in nutrient replete cells through an unknown mechanism the process of lipid droplet fatty acid liberation via cytosolic lipases (Diep et al., 2024). Importantly, both parts of the vCLIP tether complex, Ldo with its link to the lipid droplet biogenesis machinery seipin, and Vac8 with its numerous roles in the vacuole life cycle, are proteins that fulfill more than one function. This indicates that vCLIPs are integrated into a tight network of organelle communication routes, and that the vCLIP tethers likely act as hubs for the coordination of different cellular metabolic programs. It will be exciting to mechanistically understand how vCLIPs are rewired in response to environmental cues, and how these processes ultimately allow for coordinated cellular metabolic responses.

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Author Contributions

Conceptualization: D.T.V.D and M.B.; investigation: D.T.V.D.; writing – original draft: D.T.V.D and M.B.; writing – review & editing: D.T.V.D and M.B.; visualization: D.T.V.D; supervision and funding acquisition: M.B.

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