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Stationary phase lipophagy as a cellular mechanism to recycle sterols during quiescence

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Delivery of cellular contents to yeast vacuoles/mammalian lysosomes via autophagy ensures long-term cell survival and extends life span. When cultured yeast cells are grown for a prolonged period of time to enter stationary phase, a nondividing state mimicking quiescence, vacuolar membrane proteins partition into either one of the vacuolar microdomains, liquid-ordered (Lo) or liquid-disordered (Ld). We show that during the transition to stationary phase, lipid droplets (LDs), organelles originated from the endoplasmic reticulum (ER), undergo lateral movement to reach the vacuolar surface and are confined within the specific Lo microdomain underlying the network of vacuolar quasi-symmetrical micodomains. Stationary phase lipophagy uses the autophagy machineries to modify the sterol-enriched Lo microdomain to engulf LDs and subsequently deposits the LD-containing vesicles inside the vacuole lumen, which is a pathway morphologically resembling microautophagy. Moreover, stationary phase lipophagy supplies quiescent yeast cells with sterols to sustain phase partitioning of lipids for vacuolar microdomain maintenance. A feed forward loop model was proposed to depict that the sterols boosted by LDs via stationary phase lipophagy promote the Lo microdomain maintenance that in turn stimulates lipophagy.

Keywords: lipid droplets (LDs), lipophagy, liquid-ordered, quiescence, stationary phase, sterol, sterol ester, vacuolar microdomains

Abbreviations: ER, endoplasmic reticulum; LDs, lipid droplets; Lo, liquid-ordered.

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Lipid droplets are ubiquitous in eukaryotes, and the neutral lipids, triacylglycerol and sterol esters, stored within LDs supply cells with an energy source and membrane building blocks. This unique feature has placed LDs at the center of cellular lipid metabolism, which has intrigued biologists for decades. Recent conceptual changes have made LDs the organelles specialized for lipid metabolism in cell biology. Accumulating evidence indicates that molecules associated with LDs might be involved in diverse cellular activities, which further directs LD research into multiple foci. LDs emerge from the outer leaflet of the ER and stay in close proximity to the ER for most of the time. Although the exact mechanisms of LD biogenesis and maintenance remain unclear, it is conceivable that the number, size, and distribution of LDs vary under different growth conditions.

LDs are highly dynamic in varying cell types and are capable of interacting with most, if not all, organelles, probably to metabolize their neutral lipid contents. As an attempt to learn about LD content mobilization, we focus on LD dynamics of yeast cells in various growth conditions and find that LDs move gradually from the ER to the vacuole surface after the onset of the diauxic shift, the beginning of the glucose exhausted stage. When cell physiology further proceeds to the stationary phase, also known as quiescence, cell division ceases and many LDs at this stage are deposited inside the vacuole lumen along with a unique hydrolase-resistant membrane of unknown identity. Three important questions immediately arise from this observation: what is the mechanism responsible for LD translocation into vacuoles, what is the source of membrane that enwraps LDs, and what is the significance of this phenomenon?

The LD-containing vesicles accumulated in the vacuole lumen of quiescent cells morphologically resemble autophagic bodies found in protease-deficient yeast cells. To tackle the potential involvement

of autophagy, we took advantage of the well-characterized autophagy mutants in yeast. Our findings confirm that the accumulation of internal vesicles harboring LDs during stationary phase requires all of the core autophagy machineries, including those involved in autophagic induction, vesicle expansion and completion, and membrane retrieval. As this type of lipophagy is unique to the state of cells entering quiescence, we name the process "stationary phase lipophagy."

An imaging approach was taken to examine the contact sites between LDs and the vacuole limiting membrane. Surprisingly, LDs on the vacuolar surface only associate with regions that are not stained by the lipophilic dye FM 4-64. In fact, the pioneer study by Will Prinz's laboratory has established that vacuolar microdomains form when cells enter stationary phase. We show that LDs at this stage only make contact with the sterol-enriched, liquid-ordered, vacuolar microdomains labeled with Gtr2 and Ivy1, but not with the liquid-disordered vacuolar microdomain labeled with Vph1. Taking advantage of several vacuolar microdomain-deficient mutants, we confirmed the necessity of vacuolar microdomain formation for LD translocation. As the spatiotemporal evidence further supports the conclusion that the maturation of vacuolar microdomains correlates well with the progression of stationary phase lipophagy, it seems plausible that the forming phagophore modified by autophagic components for LD engulfment is the vacuolar Lo microdomain. In fact, Vps30/Atg6 and Atg14 partition selectively into the Lo microdomain in quiescent cells, strengthening the notion that the Lo microdomain serves as the donor membrane source for stationary phase lipophagy.

One important aspect of stationary phase lipophagy is the requirement of a subset of selective autophagy proteins, namely Atg21 and Atg32, implying that this lipophagy is probably a selective form of autophagy. Intriguingly, deletion of these 2, but not other, selective autophagy components and any of the core autophagy components in cells result in losing vacuolar micodomain identity in their quiescent state, even though their vacuolar microdomains initially form at the diauxic shift. Thus, this observation implies a role for stationary phase lipophagy in promoting phase partitioning of vacuolar lipids in quiescent yeast cells. One possible explanation is that LDs might supply sterols for the forming sterol-enriched vacuolar Lo microdomain. Indeed, we further show that cells harboring triacyglycerol-only LDs, when genes responsible for sterol ester synthesis are deleted, do not form vacuolar microdomains and block LD translocation. Thus, LDs, specifically sterol esters within LDs, are beneficial to vacuolar microdomain formation and thus to stationary phase lipophagy. Quiescent yeast cells, therefore, utilize storage lipids through a feed forward loop mechanism whereby lipophagy promotes lipid phase partitioning that in turn stimulates lipophagy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.