Novel mechanisms promoting giant lipid droplet formation in oleaginous yeast

Koji Okamoto

Graduate School of Frontier Biosciences, The University of Osaka

Research objective

Lipid droplets (LDs) are organelles with a phospholipid monolayer, which has neutral lipids as major components, and are present in most eukaryotic cells. In recent years, it has become evident that LDs not only store excess lipids but are also involved in dynamic lipid metabolism, thereby playing a role in the maintenance of cell homeostasis. Moreover, much attention has recently been paid to biofuel production from LDs as a project that contributes to the United Nations Sustainable Development Goals. In particular, *Lipomyces starkeyi*, an oleaginous yeast that forms giant LDs, is a promising microorganism that can be cultured in large bioreactors. Therefore, understanding the mechanism underlying LD formation is an important and fundamental issue.

This study aimed to elucidate the mechanism of LD expansion using the oleaginous yeast *L. starkeyi* as a model. When cultured under nitrogen starvation, each *L. starkeyi* cell forms a single LD at the 24h time point, which expands to occupy more than 80% of the cytosolic content at the 72h time point. Under the same conditions, the volume of LDs in the baker's yeast *Saccharomyces cerevisiae* is less than 5% of that of the whole cell. In addition, we recently found that, under nitrogen starvation, autophagy is drastically induced in baker's yeast, whereas it is strongly suppressed in *L. starkeyi* [1)]. Moreover, our studies suggest that the ubiquitin-like protein Atg8 promotes LD formation in this oleaginous yeast. Atg8 is an evolutionarily conserved protein that is uniquely conjugated to phospholipids, acting in a myriad of processes, including autophagy, lysosomal structural/functional modulation, and inflammatory responses. Based on these findings, we focused on Atg8 proteins from both oleaginous *L. starkeyi* and baker's yeasts (LsAtg8 and ScAtg8, respectively) and investigated their role in LD formation.

Methods

A 3D-structural comparison of the amino acid sequences of LsAtg8 and ScAtg8 revealed

that there are three acidic residues on the protein surface, which are apparently not involved in autophagy, positioned as a triangle. Interestingly, these three acidic residues are completely conserved among the Atg8 homologs of different oleaginous yeasts, suggesting their involvement in LD formation. To clarify the molecular functions of these acidic residues, we constructed baker's yeast expression vectors encoding wild-type ScAtg8, wild-type LsAtg8, and ScAtg8(Ls-mut) —carrying the triangle-forming acidic residues from LsAtg8 in an inverted orientation: LsAtg8(Sc-mut)—, and transformed baker's yeast with these plasmids (in all of them, gene expression was driven by the *ScATG8* promoter). The resulting transformants were grown under nitrogen starvation conditions, and Atg8 lipidation and autophagy activities were analyzed.

Results

Atg8 lipidation was assessed using urea-containing polyacrylamide gel electrophoresis (urea-SDS-PAGE) —which separates the free and lipidated forms of the protein into two different bands— followed by western blotting using an anti-Atg8 antibody. Similar to ScAtg8, ScAtg8(Ls-mut), LsAtg8, and LsAtg8(Sc-mut) were all lipidated in baker's yeast cells. Baker's yeast strains expressing a variant of the cytosolic glycolysis enzyme Tdh3 fused with the red fluorescent protein mCherry were then generated, and their autophagic activities were analyzed by western blotting with an anti-mCherry antibody. Under nitrogen starvation, Tdh3-mCherry is delivered as an autophagic cargo to the vacuole (a lysosomal organelle in yeast) for degradation. However, mCherry is resistant to vacuolar proteases, thereby, it accumulates in its free form in a manner that correlates with the autophagic activity in the cell. Additionally, we examined the strains expressing the four Atg8 variants, and the results suggested that ScAtg8(Ls-mut), LsAtg8, and LsAtg8(Sc-mut) have 70–80% the autophagic activity of ScAtg8.

Conclusion

Our results suggest that the three triangle-forming acidic residues specific to Atg8 proteins from oleaginous yeasts are not involved in Atg8 lipidation and have no significant effects on the autophagic activity of yeast cells. Under nitrogen starvation, LD expansion occurs; however, autophagy is only induced in the oleaginous yeast *L. starkeyi*. Consequently, the inhibition of LsAtg8 lipidation led to a partial suppression of LD expansion [1)]. Taken together, these findings raise the possibility that LsAtg8 harbors both autophagic and LD-expanding activities, and that these two activities may be independent of each other and could be manipulated by targeting specific organelles and/or other proteins interacting with LsAtg8. Future studies are needed to test these hypotheses.

References

1) Duan, L., Togou, A., Ohta, K., and Okamoto, K. (2025) Mitochondria-giant lipid droplet proximity and autophagy suppression in nitrogen-depleted oleaginous yeast *Lipomyces starkeyi* cells. *J. Biochem*. **177:**15-25.