Omics analysis of degradation targets by mitochondrial autophagy

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Report:

Genetic screen using a yeast knockout library identified a novel protein Atg44 essential for mitophagy. Detailed analyses of Atg44 revealed that Atg44 is dispensable for bulk/selective autophagy other than mitophagy and that Atg44 contributes to mitophagy by functioning as a mitochondrial fission factor.

Research aims

Accumulation of dysfunctional mitochondria during energy production causes harmful effects such as impaired fermentation productivity/stress resistance in yeast and neurodegenerative disease/aging in human. Mitochondrial degradation by autophagy (mitophagy) is a widely conserved quality control system of mitochondria. When mitophagy is induced upon starvation or under stationary phase of respiratory growth in yeast, the mitochondrial outer membrane protein Atg32 is phosphorylated by casein kinase 2 and then phosphorylated Atg32 accumulates on mitochondria dependently of the adaptor protein Atg11 [1),2)]. This Atg32-accumulated site is recognized as a mitochondrial degradation target (Fig. 1). However, the fission factor required for mitophagy and the contents of mitochondrial degradation target (presence/absence of mutated mitochondrial DNA and disordered proteins, *etc*) remain unknown [3)]. The aim of this study is to reveal the molecular mechanism of mitophagy and authentic mitochondrial degradation targets by genetic, biochemical, and omics approaches.

Methods

When yeast cells expressing Idh1(Isocitrate DeHydrogenase 1)-GFP are cultured in respiratory growth medium until stationary phase, mitochondria including Idh1-GFP are degraded in the vacuole following mitophagy induction. However, GFP itself, which is stable even in the vacuole, can be detected as a mitophagy signal by western blot. Employing this system in the yeast knockout library, mitophagy-defective mutants were screened. The following analyses regarding the identified mitophagy-defective mutant (*atg44Δ*) were performed; ① Whether *atg44Δ* affects bulk/selective autophagy other than mitophagy. ② Localization analysis of Atg44 using anti-Atg44 antibody. ③ Morphological analysis of mitochondria in *atg44Δ*. ④ Conservation of Atg44 in different organisms.

Results

Screen of mitophagy-defective mutants identified an unknown factor, and it was named Atg44 (AuTophaGy related 44). ① *atg44Δ* showed a strong defect in mitophagy, but not that in other autophagic pathways including bulk autophagy, pexophagy, and the Cvt pathway (Fig. 2). ② Western blot analysis using anti-Atg44 antibody detected Atg44 in a mitochondrial fraction purified from yeast cell lysates, confirming that Atg44 is a mitochondrial protein. ③ Analysis using transmission electron microscope revealed that *atg44Δ* has enlarged mitochondria, which are often observed in mitochondrial fission-deficient mutants (Fig. 3). ④ Other yeast species including the fission yeast (*S. pombe*) and methylotrophic yeast (*P. pastoris*) also possess the Atg44 orthologues, which suppress the mitophagy defect of *S. cerevisiae atg44Δ*, suggesting that Atg44 is widely conserved.

Conclusion

This study suggests that Atg44 functions as a mitochondrial fission factor essential for mitophagy. Further study using Atg44-GFP is needed to demonstrate that Atg44 indeed accumulates on the mitochondrial fission site. In addition, the proteomics analysis of the contents of mitochondrial degradation targets will be performed.

References

1) Kanki, T., Furukawa, K., and Yamashita, SI. (2015) Mitophagy in yeast: Molecular mechanisms and physiological role. *Biochim. Biophys. Acta.* **1853:** 2756-2765.
2) Furukawa, K. *et al*. (2018) The PP2A-like protein phosphatase Ppg1 and the Far complex cooperatively counteract CK2-mediated phosphorylation of Atg32 to inhibit mitophagy. *Cell Rep.* **23:** 3579-3590.
3) Yamashita, SI. *et al*. (2016) Mitochondrial division occurs concurrently with autophagosome formation but independently of Drp1 during mitophagy. *J. Cell Biol.* **215:** 649-665.

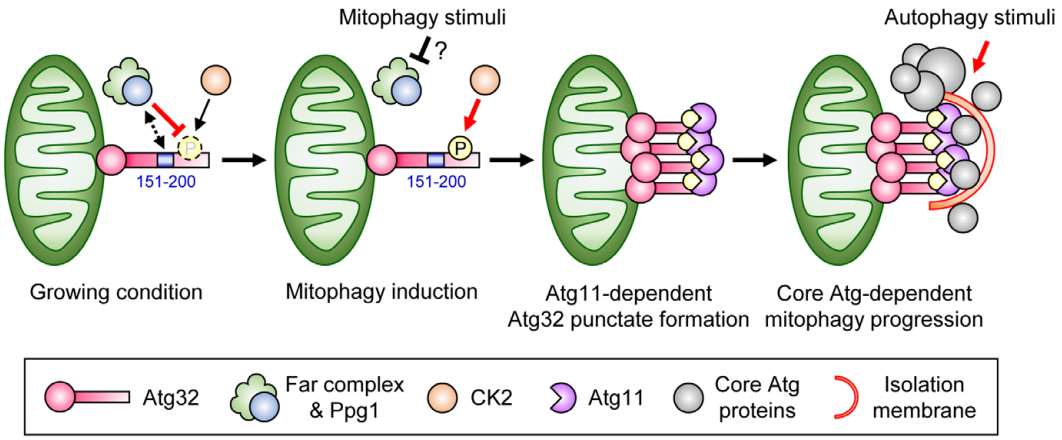

Figure 1: Model for the regulatory mechanism of mitophagy in yeast.
(Furukawa *et al.*, *Cell Rep*, 2018)

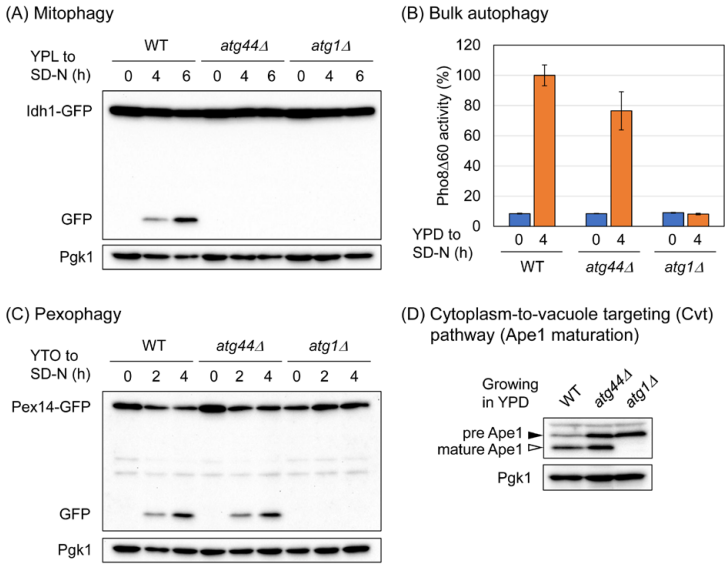

Figure 2: Atg44 is required specifically for mitophagy.
(A) Monitoring mitophagy (Idh1-GFP processing assay). (B) Monitoring bulk autophagy (alkaline phosphatase Pho80 assay). (C) Monitoring pexophagy (Pex14-GFP processing assay). (D) Monitoring the Cvt pathway (Ape1 maturation assay). *atg1D* was used as a negative control in all assays.

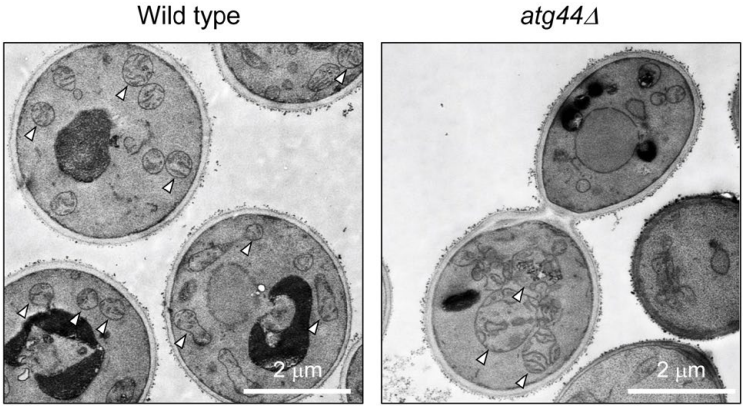

Figure 3: *atg44D* has enlarged mitochondria.
Mitochondria in wild type and *atg44D* were observed using transmission electron microscope (TEM). *atg44D* has enlarged mitochondria, suggesting that Atg44 plays a critical role in mitochondria fission. The white arrows indicate mitochondria.