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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Autophagy is a highly conserved cellular degradation and recycling system essential for maintaining cellular homeostasis in various eukaryotes. The autophagic degradation of the nucleus, the cell's administrative center, is referred to as nucleophagy and has garnered increasing attention. In the budding yeast *Saccharomyces cerevisiae*, microautophagy of the nucleus (micronucleophagy) involves the invagination of the vacuolar membrane along with the nuclear envelope at nucleus-vacuole junctions (NVJs), eventually releasing nucleus-derived vesicles enwrapped with the vacuolar membrane into the vacuolar lumen. Meanwhile, the macronucleophagy receptor Atg39 mediates the sequestration of nucleus-derived vesicles into a double-membrane vesicle called the autophagosome, which forms in the cytoplasm and then fuses with the vacuolar membrane for degradation of the contents. A previous study found that Atg39-mediated macronucleophagy is required for cell survival under nitrogen-deprivation conditions. However, how macronucleophagy contributes to cellular health remained poorly understood.

In this study, I analyzed *S. cerevisiae* cells lacking Atg39 (*atg39*Δ cells) defective in macronucleophagy in order to elucidate the causes of cell death during prolonged nitrogen starvation. I found that micronucleophagy is drastically enhanced in *atg39*Δ cells, resulting in the excessive transport of various nuclear components into the vacuole. The deletion of *NVJ1*, which is required for NVJ establishment and thus for micronucleophagy, almost fully restored viability under nitrogen starvation in *atg39*Δ cells. However, deletion of *NVJ1* not only abrogates micronucleophagy but also abolishes the NVJs. The loss of the NVJ impairs lipid transport between the nucleus and the vacuole and causes the mislocalization of NVJ proteins. Therefore, it could not be excluded that NVJ functions other than micronucleophagy contributed to cell death in *atg39*Δ cells. I successfully reconstituted NVJs that maintain normal localization of other NVJ proteins by engineering Nvj1 to interact with the vacuolar membrane protein Ivy1 or Gtr2 instead of its original binding partner Vac8. *atg39*Δ cells with Ivy1-based NVJ exhibited micronucleophagy at a level comparable to wild-type cells and accordingly maintained viability during nitrogen starvation. By contrast, Gtr2-based NVJ exacerbated cell death in *atg39*Δ cells accompanied by a greater enhancement of micronucleophagy than *atg39*Δ cells with wild-type NVJs. These findings further confirm that enhanced micronucleophagy is responsible for cell death in macronucleophagy-deficient cells.

To further understand how micronucleophagy is enhanced in *atg39*Δ cells, I demonstrated that Nvj1 and other nuclear membrane proteins localized to the NVJs are degraded through Atg39-mediated macronucleophagy. Consequently, defective macronucleophagy leads to the accumulation of these NVJ proteins. Additionally, overexpression of *NVJ1* elevates micronucleophagy and stimulates cell death during prolonged starvation. Since an increase in micronucleophagic vesicles was observed in *atg39*Δ cells, nuclear components were expected to be degraded in *atg39*Δ cells in an Nvj1-dependent manner. However, micronucleophagic vesicles were found to be long-lived in the vacuolar lumen, suggesting that cell death during prolonged starvation may be closely related to the excessive transport of nuclear components to the vacuole by enhanced micronucleophagy in *atg39*Δ cells, rather than the excessive degradation of these components.

On the other hand, a challenge that yeast cells face during prolonged starvation is medium acidification. I found that the viability of *atg39*Δ cells was maintained when cultured in pH-buffered starvation medium. My analysis to gain a deeper understanding of how enhanced micronucleophagy leads to cell death suggested that a conserved mitogen-activated protein kinase pathway, crucial for low pH response-related gene expression and cell survival under low pH conditions, was hyperactivated in *atg39*Δ cells, and was partially restored by the additional knockout of *NVJ1*. Furthermore, transcriptome analysis suggested significant alterations in gene transcription in *atg39*Δ cells during

prolonged starvation, which was cancelled by additional knockout of *NVJ1*, suggesting that enhanced micronucleophagy may affect cellular stress responses at the transcriptional level, including the alternation of low pH response-related genes.

My results revealed that macronucleophagy modulates micronucleophagy to prevent the excess removal of nuclear components partly through the degradation of the key micronucleophagy factor Nvj1, a physiological and mechanistic relationship between macro- and micro-nucleophagy. Future investigation on reconstituted NVJs may help elucidate the mechanism of vesicle formation during micronucleophagy, while analysis of transcriptional alterations in *atg39*Δ cells could further uncover which gene set(s) is directly linked to cell death during prolonged starvation.