DIVISION OF MOLECULAR CELL BIOLOGY

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This division aims to understand the physiological roles and molecular mechanism of autophagy in yeast and higher eukaryotes. All cellular activity is maintained by the balance between the synthesis and degradation of related proteins. It is now well known that the degradation process plays important roles in many physiological aspects. Autophagy is a bulk degradation system of cytosolic proteins and organelles in lysosome/vacuoles. Membrane dynamics during autophagy remain to be discovered.

I. Background

Upon nutrient starvation, the autophagic process starts as a building up of membrane structures (autophagosomes) in the cytoplasm. The autophagosome sequesters cytosol and organelles nonselectively. Then it is delivered to the vacuole/lysosome, and the cytoplasmic materials inside are degraded by vacuolar/lysosomal hydrolases. We discovered autophagy in a simple model organism, *Saccharomyces cerevisiae* and morphologically and genetically defined the whole process.

II. Hydrophobic residues in a ubiquitin-fold of Atg12 are important for autophagy in yeast

Atg12, a post-translational modifier, is activated and conjugated to Atg5 by a ubiquitin-like conjugation system, though it has no obvious sequence homology to ubiquitin. The carboxyl-terminal region of Atg12 is predicted to fold into a ubiquitin-like structure. We constructed amino-terminally truncated Atg12 mutants of the yeast *S. cerevisiae* according to the predicted secondary structure and showed that the ubiquitin-fold region of Atg12 is necessary and sufficient for both conjugation and autophagy. We also found that two hydrophobic residues within the ubiquitin-fold region are important for autophagy: mutations at Y149 affected conjugate formation catalyzed by Atg10, an E2-like enzyme, while mutations at F154 had no effect on Atg12-Atg5 conjugate formation but its hydrophobic nature was essential for autophagy. In the cells expressing F154 mutants, Atg8-PE conjugation, the other ubiquitin-like conjugation in autophagy, was severely reduced and autophagosome formation failed. A gel filtration analysis suggested that F154 plays a critical role in the assembly of a functional Atg12-Atg5·Atg16 complex requisite for autophagosome formation.

Crystal structure of *Arabidopsis thaliana* Atg12 homolog (AtATG12) has shown that it adopts the ubiquitin-like fold (Figure 1). Residues Y57 and F62, corresponding to Y149 and F154 in yeast, respectively, were found to be located adjacent to each other but form different hydrophobic patches, suggesting Atg12 utilizes two different hydrophobic patches for autophagy: one for conjugation reaction and the other for autophagosome formation.

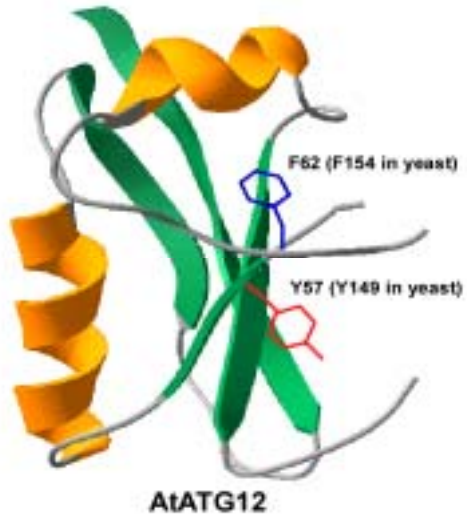

Figure 1. Two hydrophobic residues important for autophagy are located in close vicinity but form different hydrophobic patches.

III. Atg17 plays a pivotal role in autophagosome formation directed by Atg1 kinase activity

In the yeast *S. cerevisiae*, most of *ATG* genes are involved in not only the process of degradative autophagy, but also a biosynthetic process of vacuolar enzymes. In contrast, the *ATG17* gene is required specifically in autophagy. We found that the *atg17*Δ mutant under starvation condition was severely impaired in autophagosome formation. Autophagosomes with a smaller number and size were observed in the *atg17*Δ cells (Figure 2). We showed that Atg17 physically associated with an Atg1-Atg13 complex, and that it was enhanced under starvation conditions. The complex formation resulted in upregulation of an Atg1 kinase

activity, suggesting that Atg17 regulates the Atg1 kinase activity in concert with Atg13. Taken together, these results indicated that Atg17-Atg13 complex formation plays an important role in normal autophagosome formation via binding to and activating the Atg1 kinase.

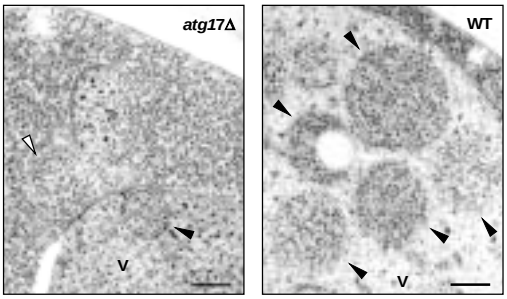

Figure 2. *ATG17* is essential for normal autophagosome formation. In a nitrogen-starvation medium, the *atg17*Δ mutant generates fewer and smaller autophagosomes (arrowheads) than those in the wild-type. Bar: 200 nm. V, vacuole.

IV. Identification and characterization of a novel autophagy-specific gene, *ATG29*

At present, at least 16 autophagy-related genes (*ATG*) have been identified. To search for novel genes involved in autophagy, we performed a genome-wide screen using a collection of ~4,500 yeast single-gene deletion mutants and identified a novel ORF, *ATG29*/*YPL166w*. *atg29*Δ cells were sensitive to starvation, and induction of autophagy was severely retarded. However, the Cvt (Cytoplasm to vacuole transport) pathway, which shares mechanistic components with autophagy, operated normally. Therefore, Atg29 is the second protein specifically required for autophagy. Furthermore we observed that an Atg29-GFP fusion protein was localized to the PAS, at which most Atg proteins are colocalized (Figure 3). From these results, we propose that Atg29 functions in autophagosome formation at the PAS in collaboration with other Atg proteins.

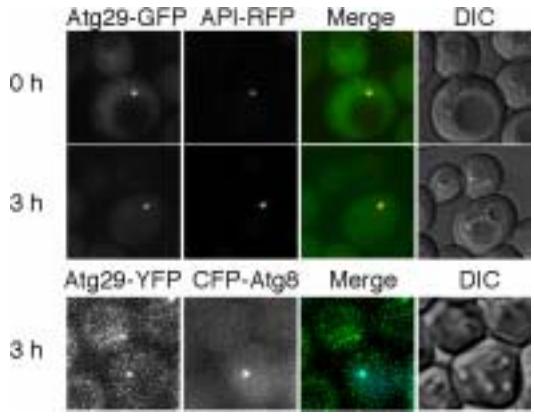

Figure 3. Localization of Atg29 at the PAS. Growing (0 h) and rapamycin treated (3 h) cells were observed by fluorescence microscopy. Atg8 and API were used as markers for the PAS.

V. Autophagy is essential for protein synthesis under nitrogen starvation

Autophagy is involved in degradation of cytoplasmic components including a significant amount of proteins, rRNA and phospholipids of organelle membranes. So far, the precise fate and the physiological importance of each degradation products generated by autophagy had been unclear. Recently, we examined changes in protein synthesis after long term nitrogen starvation and found that a heat shock protein (Hsp26p) and an enzyme involved in an amino acid biosynthesis (Arg1p) were up-regulated by nitrogen starvation in wild-type cells, but these changes did not occur in *atg* mutant cells (Figure 4A). The mRNA levels in the *atg* mutant cells were as high as those in the wild-type cells under nitrogen starvation (Figure 4B). Thus, it was suggested that synthesis of these proteins was inhibited at the translational step. We also found that bulk protein synthesis was substantially reduced in the *atg* mutant cells under nitrogen starvation compared with the wild-type cells. The wild-type cells maintained amino acid levels to survive under starvation conditions. In contrast, the total intracellular amino acid pool was reduced in the *atg* mutant cells, and the levels of several amino acids fell below critical values. These results indicate that autophagy is required to maintain physiological amino acid levels under nitrogen starvation conditions, and inability to synthesize proteins newly may explain most phenotypes in autophagy-defective mutants (e.g. inability of spore formation and so on).

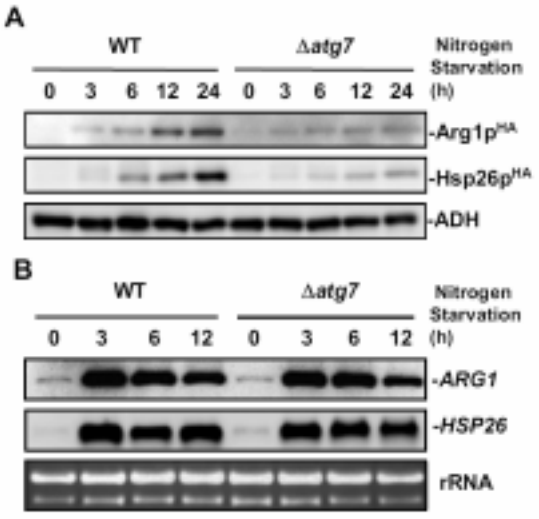

Figure 4. Autophagy is required for the expression of nitrogen starvation-induced proteins at the translational level. (A) Immuno-blot analysis of starvation-induced proteins, Arg1p and Hsp26p. (B) Northern-blot analysis of starvation-induced gene, *ARG1* and *HSP26*. ADH and rRNA were used as loading controls.

VI. Autophagy plays important roles in various aspects of plant life

By establishing an autophagy-monitoring system in a whole plant we were able show that *atatg* mutants were defective in autophagy. Our recent studies indicated that the autophagy defective mutants exhibited a reduction in the growth rate of roots under nitrogen-starved conditions and early senescence phenotype even in nutrient-rich conditions. In addition, hypersensitive response cell death was accelerated in the mutants during the plant innate immune response. These results indicate that, regardless of nutrient conditions, autophagy plays important roles in various aspects in plant life.

Publication List:

Original papers

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Suzuki, N.N., Yoshimoto, K., Fujioka, Y., Ohsumi, Y., and Inagaki, F. (2005). The crystal structure of plant ATG12 and its biological implication in autophagy. Autophagy *1*, 119-126.

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Bassham, D.C., Laporte, M., Marty, F., Moriyasu, Y., Ohsumi, Y., Olsen, L.J., and Yoshimoto, K. (2005). Autophagy in development and stress responses of plants. Autophagy, *2*, in press.