DIVISION OF MOLECULAR CELL BIOLOGY †

Professor
OHSUMI, Yoshinori

Assistant Professors:	*KAMADA, Yoshiaki*
	SUZUKI, Kuninori
	NAKATOGAWA, Hitoshi
Technical Staff:	*KABEYA, Yukiko*
Postdoctoral Fellows:	*HANADA, Takao*
	FUJIKI, Yuki
	HARASHIMA, Toshiaki
	YAMAMOTO, Hayashi
	OKAMOTO, Koji
	OKAMOTO, Noriko
	KAKUTA, Soichiro
	KOBAYASHI, Takafumi
Graduate Students:	*KAGEYAMA, Takuya*
	OH-OKA, Kyoko
Technical Assistants:	*KONDO, Chika*
	NIIMI, Kaori
	ISHII, Junko
	ICHIKAWA, Rie
Secretary:	*SUZUKI, Yuko*

Most cellular activity is maintained by the balance between the synthesis and degradation of related proteins. Degradation processes, therefore, play important roles in many physiological aspects as well as in the regulation of gene expression. Autophagy is a bulk degradation system for cytosolic proteins and organelles in lysosomes/vacuoles that is highly conserved in eukaryotic cells. Our division aims to understand the physiological roles and molecular mechanisms of autophagy in yeast and higher eukaryotes.

I. Background

In response to nutrient starvation, the autophagic process starts as a building up of membrane structures called autophagosomes in the cytoplasm. The autophagosome sequesters a part of the cytosol and organelles non-selectively. It is then delivered to the vacuole/lysosome, and the cytoplasmic materials inside are degraded by vacuolar/lysosomal hydrolases. We discovered autophagy in a simple model organism, the budding yeast *Saccharomyces cerevisiae,* and have morphologically and genetically defined the whole process.

II. Mitochondria-specific autophagy requires the transmembrane receptor Atg32

Autophagy-dependent degradation of mitochondria is a fundamental process conserved from yeast to humans. In contrast to starvation-induced, nonselective autophagy responsible for nutrient recycling, selective autophagy, which involves particular cues and receptors required for induction and cargo recognition, respectively, mediates mitochondria-specific breakdown. Although numerous studies highlight that mitochondria-specific autophagy (mitophagy) contributes to mitochondrial homeostasis, the molecular mechanisms underlying this selective clearance process are very poorly understood. We establish that a substantial fraction of mitochondria are exclusively sequestered and transported to the vacuole, a lytic compartment, in post-log phase cells under respiratory conditions (Figure 1). Using a genome-wide visual screen, we identified Atg32, a protein essential for mitophagy in budding yeast. During respiratory growth, Atg32 is highly expressed, likely in response to oxidative stress, and anchored on the surface of mitochondria. We also demonstrate that Atg32 interacts with Atg8 and Atg11, proteins critical for recognition of cargo receptors. Notably, Atg32 contains WXXI/L/V, a conserved motif that serves as a binding site for the Atg8 family members. Atg32 exposes its N- and C-terminal domains to the cytosol and mitochondrial intermembrane space, respectively. We propose that Atg32 is a transmembrane receptor that directs autophagosome formation to mitochondria.

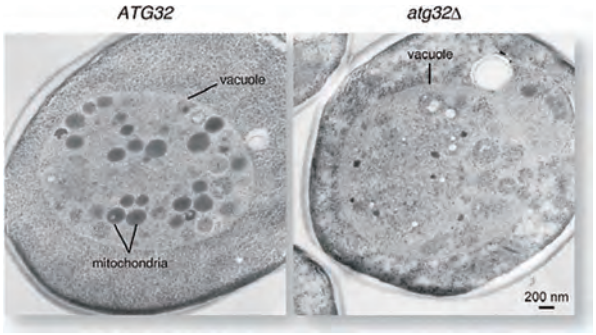

Figure 1. Electron microscopy reveals electron-dense bodies containing mitochondria accumulated in vacuoles under respiratory conditions. Vacuolar protease-deficient cells were grown in non-fermentable medium for 5 days. Notably, mitochondria are selectively incorporated into the vacuolar lumen in an Atg32-dependent manner.

III. A novel physiological role of autophagy in plants: plant autophagy puts the brakes on cell death by controlling salicylic acid signaling

It has long been thought that autophagy in plants is important for nutrient recycling and plays a critical role in the ability of plants to adapt to environmental variation such as nutrient deprivation. Recent reverse genetic studies, however, hint at other roles for autophagy, showing that autophagy defects in higher plants result in early senescence and excessive immunity-related programmed cell death (PCD), irrespective of nutrient conditions. Until now the mechanisms by which cells die in the absence of autophagy were unclear. In collaboration with RIKEN Plant Science Center research groups, using biochemical, pharmacological and genetic approaches, we revealed a conserved requirement for salicylic acid (SA) signaling for these phenomena in autophagy-defective mutants (*atg* mutants). The *atg* mutant phenotypes of accelerated PCD in senescence and immunity were suppressed by inactivation of SA signaling but not by inactivation of jasmonic acid (JA) or ethylene (ET) signalings (Figure 2). Application of an SA agonist restored the senescence/cell death phenotype in

†: This laboratory was closed on 31 March, 2009.

SA-deficient *atg* mutants but not in *atg npr1* plants, suggesting that the cell death phenotypes in the *atg* mutants are dependent on the SA signal transducer NPR1. We also showed that the SA signal can induce autophagy in plants. These findings demonstrate a novel physiological function for plant autophagy that operates a negative feedback loop to modulate SA signaling.

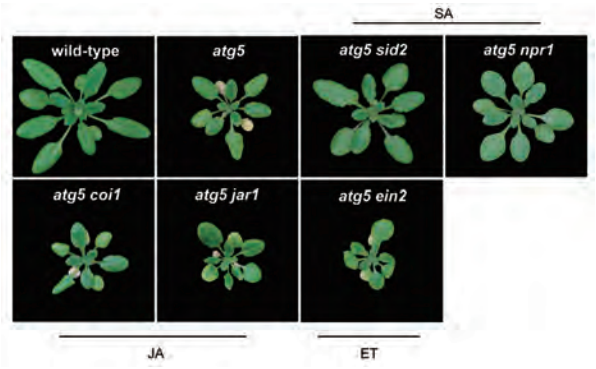

Figure 2. Accelerated PCD phenotypes in autophagy-defective plants are SA signaling dependent but do not require intact JA and ET signaling pathways. The early senescence phenotype of *atg5* mutant was suppressed by reducing SA biosynthesis in *sid2* mutant background, or by blocking SA signaling in *npr1* mutant background. Shown are photographs of five-week-old plants grown on rockwool supplied with a rich nutrient solution under long-day conditions.

IV. Characterization of the Atg17-Atg29-Atg31 complex specifically required for starvation-induced autophagy in *Saccharomyces cerevisiae*

In the yeast, *Saccharomyces cerevisiae*, Atg17, Atg29, and Atg31/Cis1 are specifically required for autophagosome formation by acting as a scaffold complex essential for pre-autophagosomal structure (PAS) organization. We show that these proteins constitutively form an Atg17-Atg29-Atg31 ternary complex, in which phosphorylated Atg31 is included (Figure 3). Reconstitution analysis of the ternary complex in *E. coli* indicates that the three proteins are included in equimolar amounts in the complex. The molecular mass of a monomeric Atg17-Atg29-Atg31 complex is calculated at 97 kDa; however, analytical ultracentrifugation shows that the molecular mass of the ternary complex is 198 kDa, suggesting a dimeric complex. We propose that this ternary complex acts as a functional unit for autophagosome formation.

V. Structural studies of Atg protein conjugation systems

Atg proteins contain two ubiquitin-like protein conjugation systems. In collaboration with Dr. Inagaki's group at Hokkaido University, understanding of these systems has proceeded from the viewpoint of structural biology. The ubiquitin-like protein Atg8 is synthesized as a precursor form with an arginine at the C terminus, which is immediately removed by the cysteine protease Atg4 to expose a glycine residue at the new C terminus. Then, this glycine of Atg8 is conjugated to the lipid PE (phosphatidylethanolamine) via reactions catalyzed by Atg7 and Atg3, E1 and E2 enzymes, respectively. Atg4 also serves as a deconjugation enzyme for Atg8; it cleaves the Atg8-PE conjugate and releases Atg8 from membranes. The crystal structure of LC3 (a mammalian Atg8 homolog) complexed with Atg4B (a mammalian Atg4 homolog) has been determined. This provided insights into

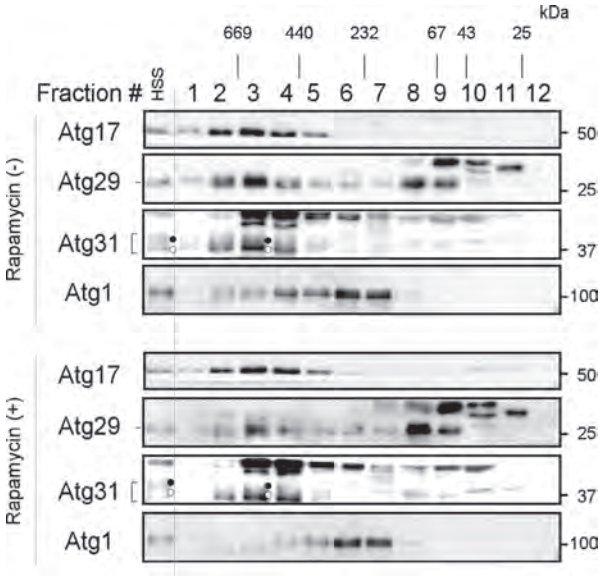

Figure 3. Atg17, Atg29, and Atg31 form a stable complex *in vivo*. Wild-type cells (BY4741) were grown and treated with rapamycin for 1 h. After cells were converted to spheroplasts, cell lysates were prepared by osmotic lysis. Cytosolic fractions (100,000 x *g* supernatant, HSS) were separated by size exclusion chromatography on a Superdex 200 column. Each fraction was analyzed by immunoblotting using anti-Atg1, anti-Atg17, anti-Atg29, and anti-Atg31antibodies. Positions of molecular mass standards (in kDa) are shown. Open and closed circles indicate dephosphorylated and phosphorylated Atg31, respectively.

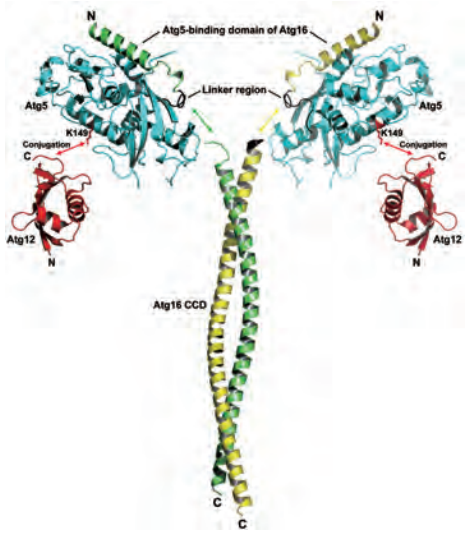

Figure 4. Model of the Atg12-Atg5-Atg16 complex.

the mechanisms of the C-terminal processing and delipidation of Atg8 by Atg4, especially for molecular recognition and regulation in these cleavage reactions.

We previously showed that the conjugation reaction of Atg8 is accelerated by a conjugate formed by the other ubiquitin-like protein Atg12 and its target protein Atg5. The Atg12-Atg5 conjugate further forms a complex with Atg16, which is required for the localization of this complex to the PAS. The crystal structure of Atg16 has been solved, which revealed that Atg16 forms a parallel coiled-coil dimer. Together with the previously-reported structures of Atg12 and Atg5, the entire architecture of the Atg12-Atg5-Atg16 complex was modeled (Figure 4). This provided an important structural basis to further elucidate the function of the complex and the mechanism of autophagosome formation.

Publication List

〔**Original papers**〕

- Hanada, T., Satomi, Y., Takao, T., and Ohsumi, Y. (2009). The amino-terminal region of Atg3 is essential for association with phosphatidylethanolamine in Atg8 lipidation. FEBS Lett., *583*, 1078-1083.
- Kabeya, Y., Noda, N.N., Fujioka, Y., Suzuki, K., Inagaki, F., and Ohsumi, Y. (2009). Characterization of the Atg17-Atg29-Atg31 complex specifically required for starvation-induced autophagy in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Comm., *389*, 612-615.
- Kageyama, T., Suzuki, K., and Ohsumi, Y. (2009). Lap3 is a selective target of autophagy in yeast, *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Comm., *378*, 551-557.
- Okamoto, K., Kondo-Okamoto, N., and Ohsumi, Y. (2009). Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev. Cell, *17*, 87-97.
- Satoo, K., Noda, N.N., Kumeta, H., Fujioka, Y., Mizushima, N., Ohsumi, Y., and Inagaki, F. (2009). The structure of Atg4B-LC3 complex reveals the mechanism of LC3-processing and delipidation during autophagy. EMBO J., *28*, 1341-1350.
- Sekito, T., Kawamata, T., Ichikawa, R., Suzuki, K., and Ohsumi, Y. (2009). Atg17 recruits Atg9 to organize the pre-autophagosomal structure. Genes Cells, *14*, 525-538.
- Shin, J.H., Yoshimoto, K., Ohsumi, Y., Jeon, J.S., and An, G. (2009). OsATG10b, an autophagosome component, is needed for cell survival against oxidative stresses in rice. Mol. Cells., *27*, 67-74.
- Wada, S., Ishida, H., Izumi, M., Yoshimoto, K., Ohsumi, Y., Mae, T., and Makino, A. (2009). Autophagy plays a role in chloroplast degradation during senescence in individually darkened leaves. Plant Physiol., *149*, 885-893.
- Watanabe, Y., Noda, N.N., Honbou, K., Suzuki, K., Sakai, Y., Ohsumi, Y., and Inagaki, F. (2009). Crystallization of *Saccharomyces cerevisiae* alpha-mannosidase, a cargo protein of the Cvt pathway. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun., *65*, 571-573.
- Yoshimoto, K., Jikumaru, Y., Kamiya, Y., Kusano, M., Consonni, C., Panstruga, R., Ohsumi, Y., and Shirasu, K. (2009). Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in *Arabidopsis*. Plant Cell, *21*, 2914-2927.

〔**Original papers (E-publication ahead of print)**〕

- Fujioka, Y., Noda, N.N., Nakatogawa, H., Ohsumi, Y., and Inagaki, F. The dimeric coiled-coil structure of *Saccharomyces cerevisiae* Atg16 and its functional significance in autophagy. J. Biol. Chem. 2009 Nov 4.
- Kamada, Y., Yoshino, K., Kondo, C., Kawamata, T., Oshiro, N., Yonezawa, K., and Ohsumi, Y. Tor directly controls the Atg1 kinase complex to regulate autophagy. Mol. Cell Biol. 2009 Dec 7.

〔**Review articles**〕

- Nakatogawa, H., Suzuki, K., Kamada, Y., and Ohsumi, Y. (2009). Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat. Rev. Mol. Cell Biol., *10*, 458-467.
- Noda, N.N., Ohsumi, Y., and Inagaki, F. (2009). ATG systems from the protein structural point of view. Chem. Rev., *109*, 1587-1598.
- Okamoto, K., Kondo-Okamoto, N., and Ohsumi, Y. (2009). A landmark protein essential for mitophagy. Autophagy, *5*, 1203-1206.