DIVISION OF BIOENERGETICS

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This division aims to elucidate the mechanism and its regulation of intracellular protein degradation in a lytic compartment. Recently it was realized that degradation process plays essential roles for various physiological function. In the cytoplasm selective protein degradation takes place by ubiquitin/proteasome system. Short-lived or abnormal proteins are selectively eliminated by this pathway. While vacuole/lysosome contributes to the bulk turnover of cytosolic and organelles proteins.

Little is known about the mechanism of protein degradation in contrast with that of biosynthesis. Bulk protein degradation is induced by various nutrient starvation condition, which is obligatory to cell differentiation and maintenance of cell viability. Autophagy is a major route for sequestration of proteins to the lytic compartment (Fig.1). Since cellular lytic compartment, lysosome, was identified, biogenesis of lysosomal enzymes and their enzymatic characterization have been studied thoroughly. However, the mechanisms of delivery of proteins to the lysosomes are not known at a molecular level. Biochemical analysis of lysosome/vacuole system is quite difficult because of its complexity and dynamic feature. Autophagic process has been studied mostly by electron microscopy, and molecular mech ıism of autophagy remains to be elucidated.

Yeast Induces Autophagy as Mammalian Cells

Recently we discovered yeast, *Saccharomyces cerevisiae*, induces bulk protein turnover in the vacuoles under starvation conditions. This whole process corresponds to that of macroautophagy in higher eukaryotic cells. By electron microscopic analyses we succeeded in detecting double membrane structures in the cytoplasm enclosing a portion of cytosol. These yeast autophagosomes immediately fuse with vacuoles, delivering single inner membrane structures, autophagic bodies in the vacuole. When vacuolar proteinase activities are blocked genetically or by specific inhibitor such as PMSF autophagic bodies are accumulated in the vacuoles. They moves around vigorously in the vacuoles by Brownian motion, and are easily detectable by light microscope. Thus we can follow the progression of autophagy as the accumula-

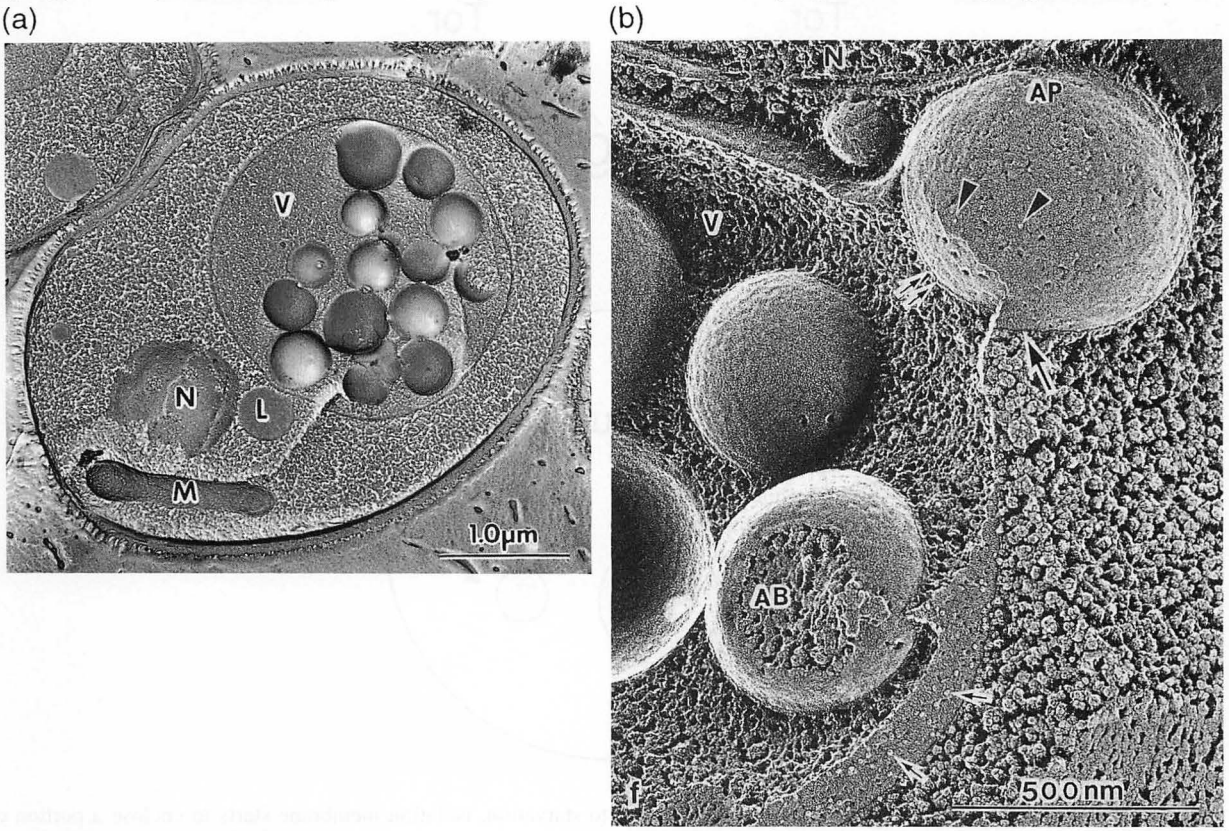

Fig. 1. (a) Freeze-Fracture Image of Yeast under Nitrogen Starvation.
When vacuolar proteinases are blocked, spherical membrane structures, autophagic bodies accumulate in the vacuole.
(b) Fusion of Autophagosome to the Vacuolar Membrane.
Double membrane structure, autophagosome (AP), encloses a portion of the cytosol, then fuses with the vacuole to deliver an autophagic body (AB) in the vacuole. These membrane structures show very few intramembrane particles.

tion of autophagic bodies in real time. Biochemical and immunoelectron microscopic analyses of vacuoles containing autophagic bodies showed that starvation-induced sequestration is non-selective, that is, any kind of cytosolic enzymes and organelles are sequestered randomly to the vacuoles to be degraded.

Autophagy in yeast is a kind of general stress response to adverse environmental condition and is induced by various starvation conditions such as nitrogen, carbon, sulfate, and even single amino acid depletion. Signal transduction of starvation for induction of autophagy is a key question to be solved. Recently we found rapamycin induces autophagy in the cell growing in a rich medium and that Tor, phosphatidyl inositol kinase homologue plays essential role for the regulation of autophagy (Fig.2).

Another crucial and controversial question is the mechanism of formation of autophagosome. Still nothing is clear about the origin of the membrane. Freeze-fracture electron microscopy showed that autophagosomal membrane has quite characteristic feature and the density of intramembrane particles is extremely low as compared with other intracellular membrane.

Genetical dissection of autophagic process

Yeast, *S. cerevisiae*, has made great contribution to solve many fundamental problems in cell biology because of tractability of genetic and molecular biological techniques. In order to dissect the complex process of autophagy to its elementary steps we started to isolate mutants in the process of autophagy, taking advantage of morphological selection under light microscope. Total 14 autophagy defective mutants, *agp*, were isolated. They cannot induce protein degradation upon shift from growth medium to the starvation medium. They grow normally in a rich medium, but start to die after 2 days in the starvation medium. This suggests that the autophagy is necessary for long time maintenance of cell viability. Homozygous diploid of each *apg* mutant is sporulation-defective as expected.

Analyses of *APG* genes

Now we are focusing on characterization of these *APG* genes, essential for autophagy. So far we have finished cloning and identification of twelve *APG* genes. *APG1* codes a novel Ser/Thr protein kinase essential for the induction of autophagy. This pro-

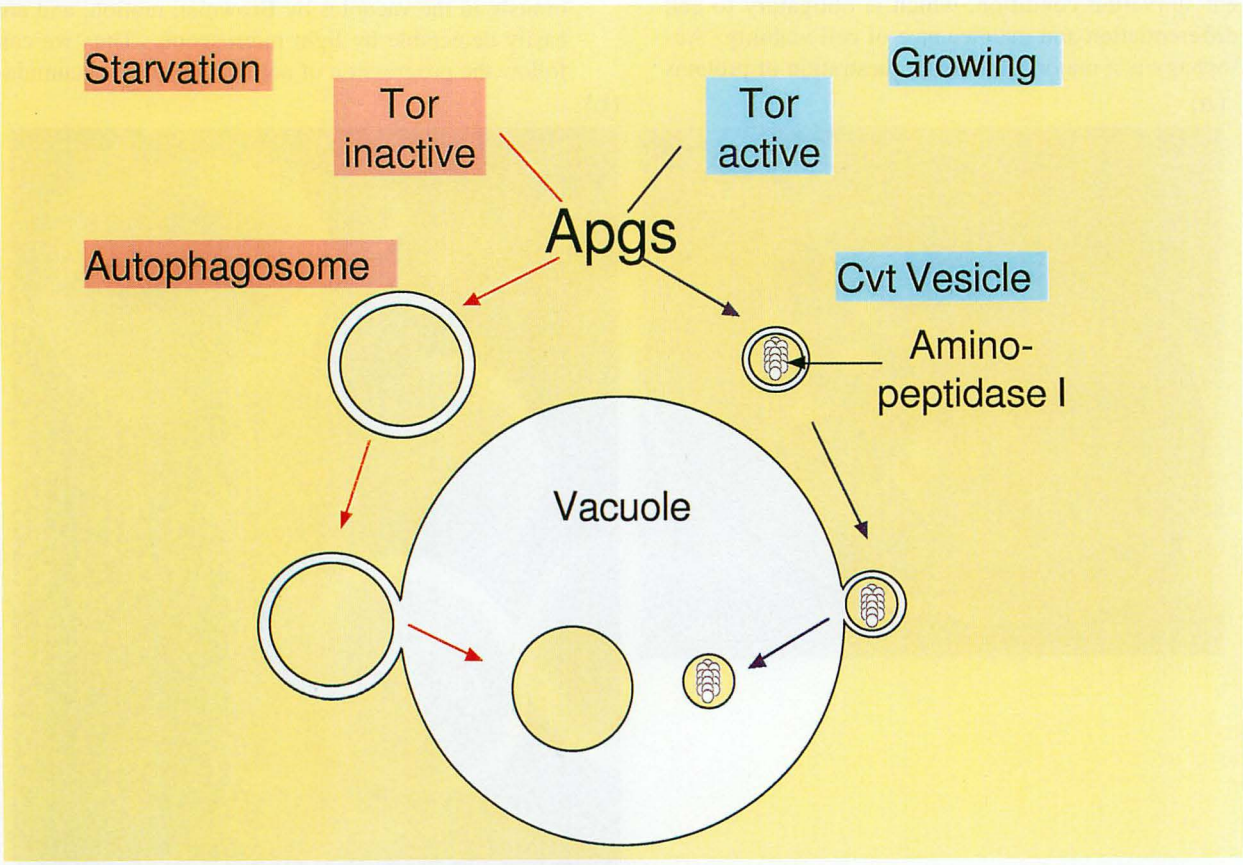

Fig. 2 Schema of autophagy in yeast. When yeast cells face to starvation, isolation membrane starts to enclose a portion of cytosol and form an autophagosome. Autophagosome fuses to the vacuolar membrane, delivering autophagic body in the vacuoles (Autophagic pathway). Under growing condition one of vacuolar enzymes, API is selectively sequestered to the vacuole (Cvt pathway). Two distinct pathways share most molecular machinery.

vides the first direct evidence for involvement of protein phosphorylation in the process of autophagy. Apg13p interacts with Apg1p and might function as a regulator of its kinase activity. So far most *APG* genes analyzed are novel and non-essential for vegetative growth. Using the assay system of autophagy we developed, we are studying genetic interaction among *APG* genes. Some *APG* gene products are inducible or change phosphorylation state by nutrient starvation. Analyses of intracellular localization of these Apgps may provide us specific marker to elucidate the membrane dynamics during autophagy.

Diverse pathways of autophagy

Autophagy is ubiquitous and fundamental physiological response of every eukaryotic cell. We found yeast cells induce macroautophagy under starvation, which is a main route for bulk and non-selective protein degradation. While it is known that excessive amount of organelles are also degraded by autophagic process. In methylotrophic yeast, *Pichia pastoris* degrades peroxisomes by invagination of vacuolar membrane, microautophagic process. Further studies will uncover more sophisticated pathways for the degradation in the lysosome/vacuoles.

Recently it was found that all *APG* genes are required for targeting of one of vacuolar enzyme, API, from cytosol to the vacuole. Electron microscopic analyses of API transport process revealed that API first forms a complex in the cytosol and delivered to the vacuole by the distinct but topologically similar mechanism to the macroautophagy (Fig.2). However, API transport is selective and constitutive process. It is interesting that biosynthetic pathway shares the machinery with degradative process. Some selective degradation may also be mediated by autophagy-related mechanism.

Perspective

Autophagy is essential for maintenance of cell viability during starvation. Degradation products may provide essential nutrients necessary for minimal protein synthesis, or reduction of certain critical activities in the cytosol may be essential for the maintenance of viability. Molecules involved in the membrane dynamics are well conserved from yeast to higher eukaryotes. We realized some *APG* genes in yeast show homologues in mammals or higher plants. Knowledge in yeast must give us key to uncover the mechanism of autophagy in higher eukaryotic organisms. We are now developing systems for studying autophagy in mammalian cell culture.

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