DIVISION OF BIOENERGETICS

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This division aims to elucidate the mechanism and regulation of intracellular protein degradation in a lytic compartment and its physiological function. Recently it was realized that degradation process plays essential role for cellular regulation. 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. However, little is known about the mechanism of protein degradation in contrast with 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. Vacuole/lysosome is also the destination for endocytic pathway. Since in 1952 du Duve identified cellular lytic compartment, lysosome, enzymatic characterization and biogenesis of lysosomal enzymes have been studied thoroughly. However, the mechanisms of delivery of proteins to the lysosomes are not known at a molecular level. Dynamism of lysosomal system has been studied mostly with electron microscope. Autophagy is one of the most important problems in cell biology remained to be solved.

Yeast Induces Autophagy as Mammalian Cells

Recently we discovered yeast, *Saccharomyces cerevisiae*, induces bulk protein turnover in the vacuoles under various starvation conditions. This whole process corresponds to the process of macroautophagy in higher eukaryotic cells. By electron microscopic analyses we succeeded to detect double membrane structure enclosing a portion of cytosol in the cytoplasm. These yeast autophagosomes immediately fuse with vacuolar membrane, resulting to deliver a single membrane bound vesicles, autophagic bodies in the vacuoles. When vacuolar proteinase activities are blocked genetically or by specific inhibitor such as PMSF autophagic bodies are accumulated in the vacu-

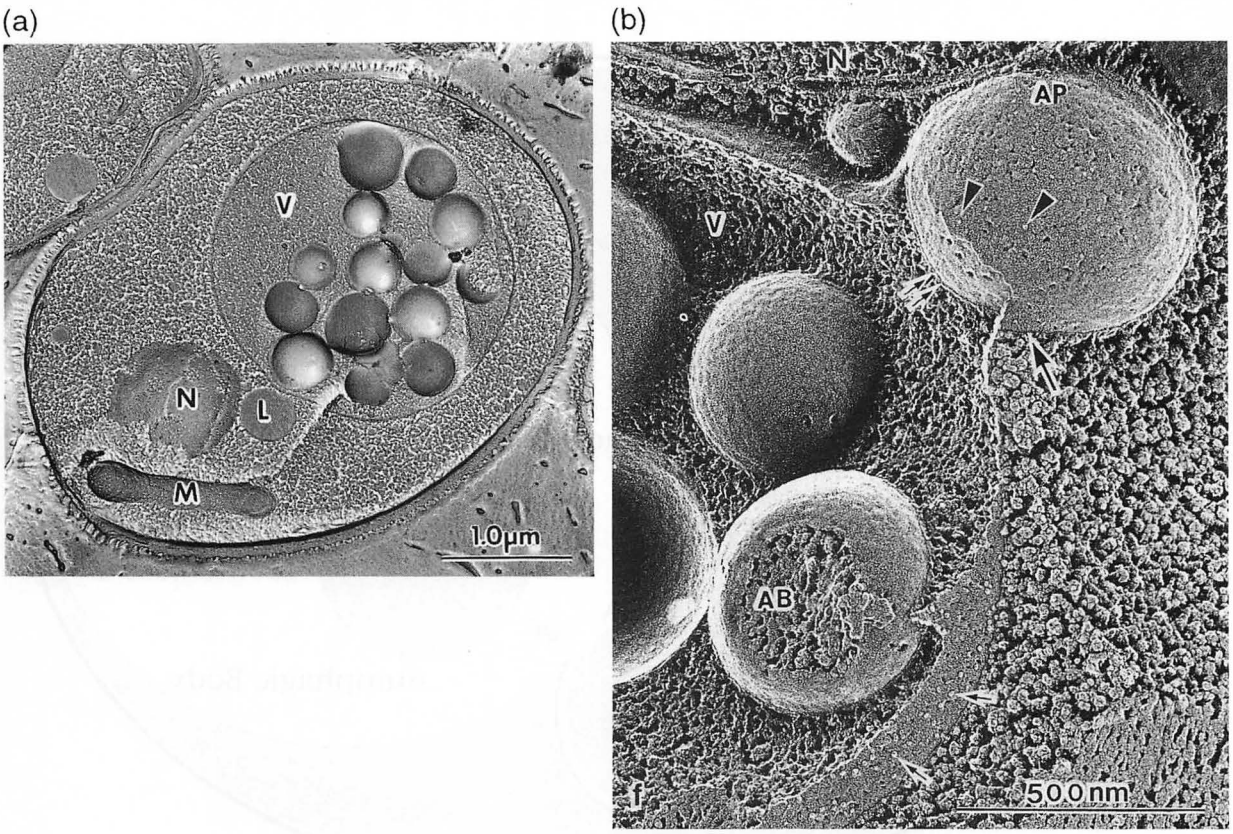

Fig. 1. (a) Freeze-Fracture Image of Yeast under Nitrogen Starvation.
When vacuolar proteinases are blocked, spherical membrane structure, autophagic bodies accumulate in the vacuole.
(b) Fusion of Autophagosome to the Vacuolar Membrane.
Double membrane structure, autophagosome, encloses a portion of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

oles. They moves around vigorously in the vacuoles by Brownian motion, and are easily observed by light microscope. Thus we can follow the progression of autophagy by the accumulation 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 to the vacuoles to be degraded. Freeze-fracture electron microscopy showed that autophagosomal membrane has characteristic feature and density of intramembrane particles is quite few. The origin of the autophagosomal membrane and mechanism of formation are key problems remained to be solved.

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 *agp* mutants, defective in the induction of autophagy, were isolated. They cannot induce protein degradation upon shift from growth medium to the starvation medium. But 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* mutants is sporulation defective as expected.

Analyses of *APG* genes

Now we are focusing on characterization of these *APG* genes, essential for autophagy. This year we have finished cloning of twelve *APG* genes. So far ten *APG* genes have been identified. *APG1* codes a novel Ser/Thr kinase essential for the induction of autophagy. This provides the first direct evidence for involvement of protein phosphorylation in the process of autophagy. So far all genes analyzed are novel and non-essential for vegetative growth. Using the assay system of autophagy we developed, we are now analyzing genetic interaction among *APG* genes. Further molecular biological and biochemical studies on these *APG* gene products might provide important clues to understand the mechanism of autophagy at a molecular level. Now we obtained antibodies against to these *APG* gene products. Intracellular localization of them may give us clues to elucidate the function of these proteins.

Diverse pathways of autophagy

Autophagy is one of the fundamental function of all eukaryotic cells. We found yeast cells induce macro-

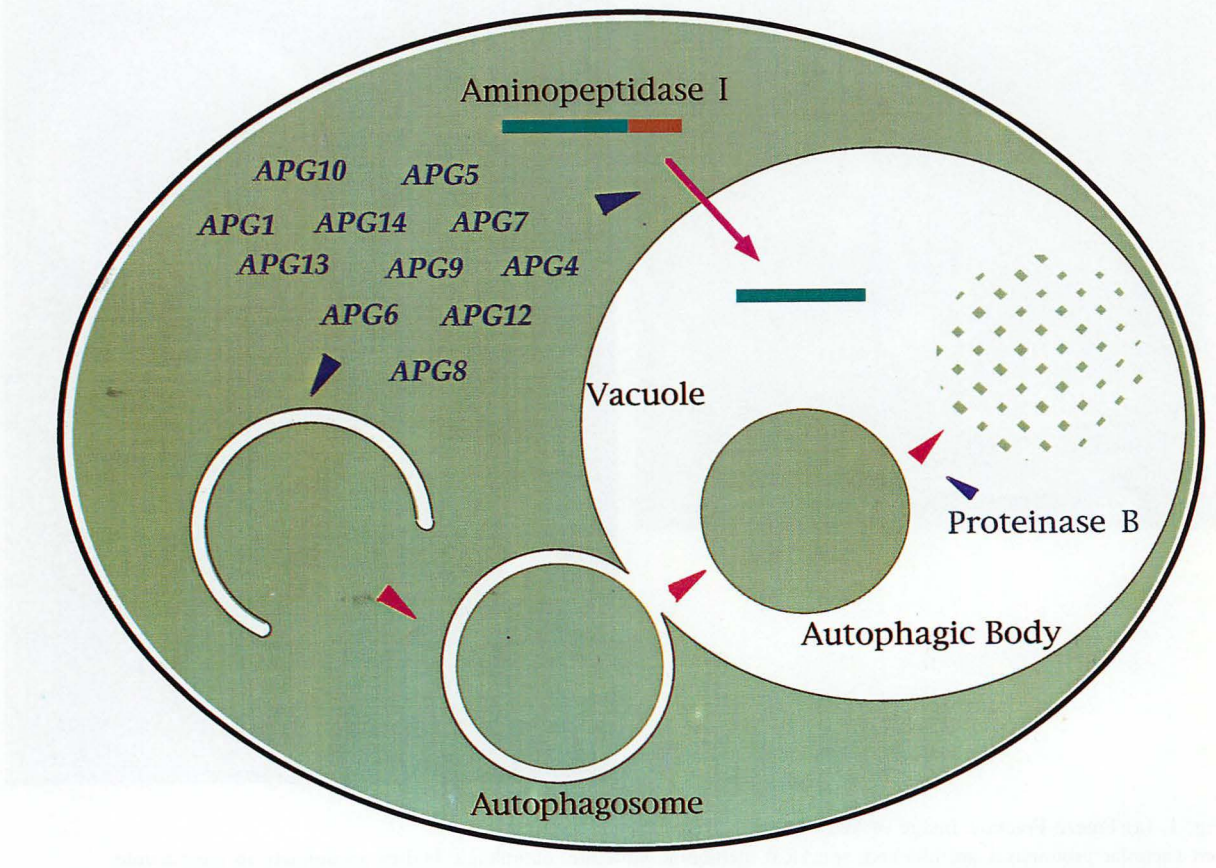

Fig. 2. Scheme of Autophagy in Yeast
All *APG* genes identified are novel and required at or before the formation of autophagosome.

autophagy under starvation, which is a main route for bulk and non-selective protein degradation. However, it is also reported that some enzymes are selectively taken up to vacuoles according to the physiological demands. 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 lysome/vacuoles. Recently it was found that all *APG* genes are reuired for targeting of API from cytosol to the vacuole. It is interest problem how this selective and constitutive process shares machinery with the autophagy.

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. Basic molecular devices for the membrane dynamics are conserved from yeast to higher eukaryotes. We realized some *APG* genes in yeast show homologues in mammals or higher plants. We are now developing systems of autophagy in mammalian cultured cell.

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