【Grant-in-Aid for Scientific Research (S)】
Biological Sciences (Biology)

Title of Project : Molecular mechanism and physiological understanding of Autophagy

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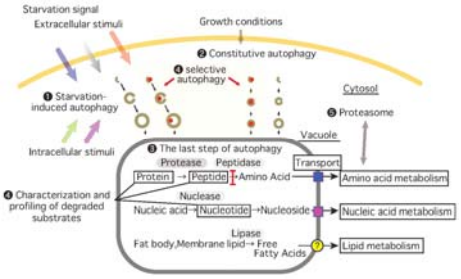
Research Project Number : 16H06375 Researcher Number : 30114416
Research Area : Molecular Cell Biology
Keyword : autophagy・proteolysis・RNA degradation・ATG・yeast

【Purpose and Background of the Research】
Autophagy is a fundamental degradative pathway that occurs within the cell's own lytic compartment (the vacuole or the lysosome) and is conserved throughout virtually all eukaryotic cells. A detailed description of autophagy is therefore indispensable for a complete understanding of the basic unit of life, the cell. Lending on the 27 years of experience of the leading researcher, this project will use yeast as a model organism to undertake a systematic and rigorous biochemical interrogation of the yet-uncharacterised physiological functions of autophagy, providing a comprehensive picture of the role of autophagy in the cell.

【Research Methods】
1. Understanding the conditions of autophagy induction. We aim to describing the induction of autophagy under a range of nutrient rich and deplete conditions, with a particular emphasis induction during starvation for zinc and iron and the physiological role of autophagy as a response to such conditions.
2. Uncovering the mechanism of autophagy induction during carbon-source starvation We have found that as part of their response to carbon deprivation, yeast induce autophagy when grown on non-fermentable sources of carbon. We will investigate the induction signals and identify molecular substrates of autophagy under these conditions.
3. Establishment of an analytical regime for autophagic proteolysis. We will generate strains of yeast lacking all nine amino-and carboxyl-peptidases of the vacuole, and investigate the phenotypic outcomes of this manipulation under starvation conditions. Using these mutants, autophagy-derived peptides that accumulate in the vacuole will be assessed by biochemical and cell biology analytical techniques. In addition, we will identify proteins degraded by autophagy under a range of conditions using mass spectrometric analyses of these peptides. The establishment of this analytical regime is therefore also a priority in our work.
4. Examination of the degradation of RNA by autophagy We will examine vacuolar degradation of RNA and elucidate enzymes connected to nucleotide metabolism in the cell. This involves the establishment of a comprehensive experimental regime that is able to identify specific substrates of autophagic RNA degradation. At the outset, we will examine the degradation of rRNA, tRNA, ncRNA and others, before examining the meaning of this degradation. In this project, we also aim to develop highly-sensitive assays for the detection of modified bases and nucleotides released from the cell, enhancing the potential for autophagy as a quantitative indicator of cellular processes.
5. Other lines of enquiry. We will also undertake analyses of the mechanism of autophagic secretion, the mechanistic link between autophagy and the initiation and arrest of cell growth, constitutive autophagy and other projects related to the physiological role of autophagy in the cell.



【Expected Research Achievements and Scientific Significance】
Autophagy is currently garnering the most attention of the diverse fields of cell biology. Divergent physiological roles of autophagy have been uncovered, but their explicit implications remain unclear. One reason why is because biochemical analysis of the lysosome is difficult. Using the unique features of the yeast vacuole, we aim to address the important questions of what, when and how autophagy degrades cellular components in the cell, which are key to the continued development of this important field and our understanding of cell biology.

【Publications Relevant to the Project】
1.Takeshige, K., and, Ohsumi Y et.al Autophagy in yeast demonstrated with proteins-deficient mutants and its conditions for induction. J. Cell Biol., 119, 301-311 (1992)
2.Tsukada, M., and Ohsumi, Y. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett., 333, 169-174 (1993)
3.Mizushima, N., and Ohsumi, Y.et. al A protein conjugation system essential for autophagy. Nature, 395, 395-398 (1998)
4.Nakatogawa, H. and Ohsumi Y et.al Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. Cell, 130, 165-178(2007)
5.Huang H*, Kawamata T*, Ohsumi Y**, Fukusaki E**et.al. Bulk RNA degradation by nitrogen starvation-induced autophagy in yeast. EMBO J. 34, 154-168 (2015)

【Term of Project】FY2016-2020
【Budget Allocation】143,700 Thousand Yen
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