【Grant-in-Aid for Young Scientists(S)】
Biological Sciences (Biology)

Title of Project：Molecular mechanism of autophagosome formation in mammalian cells

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Research Area：Biology

Keyword：Protein degradation, intracellular organelle

【Purpose and Background of the Research】

Autophagy is the primary means for the degradation of cytoplasmic constituents in the lysosome (Fig. 1). When autophagy is induced, a portion of cytoplasm is sequestered by autophagosomes, and then delivered to lysosomes. Autophagy is important for many physiological processes such as starvation adaptation, intracellular quality control, early embryogenesis and antigen presentation. While its physiological roles have been rapidly identified, the mechanism of autophagosome formation remains to be understood. Although it has been extensively studied in yeast, recent identification of mammalian homologs of the yeast autophagy-related (Atg) genes and establishment of various knockout/knockdown cells now allow us to perform systematic analysis of autophagosome formation in mammals. There may be mammalian-specific factors, which are absent in yeast. In this research, we will dissect autophagosome formation process by morphological and biochemical methods using mammalian cells defective in various Atg genes. These studies will provide new insights into the origin of autophagosome and mechanism of autophagosome formation.

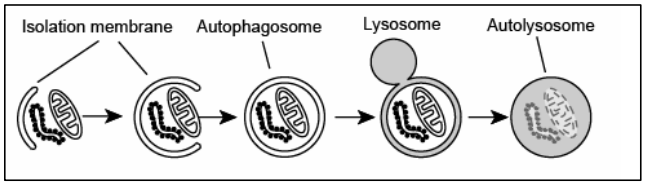

Fig. 1. Scheme of autophagosome formation

【Research Methods】

1. Analysis of the ULK1 complex

(m)TOR is known to negatively regulate autophagy both in yeast and mammalian cells. We recently found that ULK1, mAtg13 and FIP200 form a huge protein complex, which localizes to isolation membrane. Under nutrient rich conditions, mTORC1 associates with the ULK1-mAtg13-FIP200 complex and phosphorylates ULK1 and mAtg13. To better understand the early stage of autophagosome formation, we will therefore isolate additional components included in this complex and identify potential substrates of ULK1 kinase.

2. Morphological and biochemical dissection of autophagosome formation.

Mammalian Atg proteins are now classified into five functional modules. Using cells defective in each module, we will study the hierarchical relationships among these Atg proteins and dissect autophagosome formation at the molecular levels. We will try to isolate precursor and intermediate structures of autophagosome formation and analyze their molecular composition.

【Expected Research Achievements and Scientific Significance】

These studies will be of help to understand diversity and selectivity of autophagy, which are recent topics in this research field. Furthermore, results of these studies will be applied for development of new autophagy-monitoring methods, and identification of therapeutic targets.

【Publications Relevant to the Project】

Hosokawa N, Hara T, *Mizushima N et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. ***Mol. Biol. Cell*** 20: 1981-1991 (2009)

Itakura E, Kishi C, Inoue K, Mizushima N. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. ***Mol. Biol. Cell*** 19: 5360-5372 (2008)

Hara T, Takamura A, Kishi C, Iemura S, Natsume T, Guan JL, Mizushima N. FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. ***J. Cell Biol.*** 181: 497-510 (2008)

【Term of Project】 FY2009-2013

【Budget Allocation】 80,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.tmd.ac.jp/med/phy2/phy2-E.html