Dissection of ESCRT-mediated microautophagy induction after TORC1 inactivation in budding yeast

（課程博士・様式７）(Doctoral qualification by coursework, Form 7)

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：
Title of Thesis：**Dissection of ESCRT-mediated microautophagy induction after TORC1 inactivation in budding yeast**

論文要旨：

Abstract：
Autophagy is a main bulk degradative process of proteins and intracellular organelles in lysosomes/vacuoles. For maintaining cellular homeostasis, superfluous or damaged proteins and organelles should be eliminated. Degraded molecules are utilized for macromolecular synthesis and energy source, which is essential in nutrient-starved conditions. In microautophagy, a mode of autophagy, vacuolar/lysosomal membranes directly engulf the cytoplasmic cargo. Vacuolar membrane proteins together with vacuolar membranes are degraded in the vacuole in the course of microautophagy. Overall microautophagy flux has been estimated using GFP-tagged vacuolar transmembrane proteins Vph1 and Pho8. When these GFP-tagged proteins are incorporated into the vacuole by microautophagy, Vph1 and Pho8, but not the stable GFP moiety, are degraded by vacuolar proteases, producing free GFP, which is detectable by immunoblotting. Microautophagy is induced after nutrient starvation and inactivation of target of rapamycin complex 1 (TORC1) protein kinase. Diseases related to microautophagy and chaperon-mediated autophagy are less understood.

Microautophagy requires the endosomal sorting complex required for transport (ESCRT) that promotes vacuolar membrane invagination, constriction, and fission of membranes. The ESCRT complexes are combined with five individual ESCRT complexes (ESCRT-0, -I, -II, -III and the Vps4 complexes). ESCRT-0 is first recruited onto the membrane via association with ubiquitinated membrane proteins, and clusters the ESCRT-I and -II complexes, promoting the assembly of ESCRT-III, which promotes membrane deformation. ESCRT-0 consists of two subunits, Vps27 (vacuolar protein sorting 27) and Hse1 (Has symptoms of class E mutants 1) (Hrs and STAM1/2 in

human). Vps27 binds to phosphatidylinositol 3-phosphate (PI3P) via a FYVE (Fab1, YGL023, Vps27, and EEA1) domain, and to ubiquitin conjugated with membrane proteins via two ubiquitin-interacting motif (UIM) domains and a Vps27, Hrs and STAM (VHS) domain. In contrast, Hse1 has an UIM domain and a VHS domain, but not a FYVE domain. All of the five ubiquitin-binding sites in ESCRT-0 are required for MVB formation. TORC1 promotes phosphorylation of Vps27 and TORC1 inactivation facilitates recruitment of Vps27 onto the vacuolar membrane, probably facilitating ESCRT-mediated microautophagy induction. However, whether and how TORC1 regulates other factors of ESCRT in the context of microautophagy is unknown.

In Chapter 2, I showed that Hse1 is also recruited onto vacuolar membranes after TORC1 inactivation, promoting formation of ESCRT-0 complex on vacuolar membranes. Hse1 recruitment was dependent on Vps27, whereas Vps27 recruitment was independent of Hse1. Not only Vps27 but also Hse1 was required for ESCRT-III recruitment onto vacuolar membranes and microautophagy induction after TORC1 inactivation. This study revealed that ESCRT-0 (Vps27–Hse1) complex formation on vacuolar membranes is important for microautophagy inactivation after TORC1 inactivation.

In Chapter 3, I provide evidence that the AGC protein kinase (protein kinase A, G and C)-family kinase Sch9 mediates ESCRT-mediated microautophagy after TORC1 inactivation. Sch9 was required for recruitment of Hse1, but not Vps27, onto vacuolar membranes, ESCRT-0 formation on vacuolar membranes, ESCRT-III recruitment onto vacuolar membranes and microautophagy induction after TORC1 inactivation. Unexpectedly, hypoactive, but not hyperactive, Sch9 is required for microautophagy induction and Hse1 recruitment. I found evidence that Sch9 regulated the phosphorylation status of Hse1. This study revealed that the TORC1–Sch9–Hse1 axis is critical for ESCRT-mediated microautophagy inactivation.

The molecular mechanism of ESCRT-mediated microautophagy is still largely mysterious in yeast and humans. This study revealed that the TORC1–Sch9–Hse1 axis promotes ESCRT-mediated microautophagy. I would believe that this study provides a novel insight into involvement of protein kinase signaling in microautophagy induction and therapeutic treatments of microautophagy-related diseases.