Analysis of autophagy induction in budding yeast

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

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Abstract：

Autophagy degrades cytoplasmic components and intracellular organelles in lysosomes/vacuoles, which is a conserved system from the yeast to mammalian cells. Newly generated cup-shaped membranes, called isolation membranes, expand to encapsulate cellular constituents and then the edges of isolation membranes fuse to form double membrane-surrounded autophagosomes. Subsequently, autophagosomes fuse with lysosomes/vacuoles, and the engulfed cargoes are digested by lysosomal hydrolytic enzymes. The breakdown products are transported back into the cytoplasm and are reused in the cells. The pre-autophagosomal structure (PAS) proximal to the vacuole is a putative site for autophagosome formation in the budding yeast *Saccharomyces cerevisiae*. Most autophagy related Atg proteins are localized to the PAS.

In response to nutrient starvation, autophagy is dramatically induced to recycle proteins and other cellular components. Target of rapamycin complex 1 (TORC1), a nutrient-responsive protein kinase, regulates autophagy induction. TORC1 phosphorylates Atg13 in nutrient-sufficient conditions, but TORC1 is inactivated in nutrient-starved conditions or by the specific TORC1 inhibitor rapamycin, which causes Atg13 dephosphorylation. Dephosphorylated Atg13 forms a complex, called the Atg1 kinase complex, consisting of Atg1 kinase, Atg13, Atg17, Atg29 and Atg 31. Formation of the Atg1 complex is required for Atg1 kinase activation and for PAS formation and thereafter autophagy induction. Thus, TORC1 is the key protein kinase to regulate autophagy. However, the phosphatases involved in Atg13 dephosphorylation and PAS formation is largely unknown at present.

Here I screened phosphatases involved in PAS induction and autophagy induction after TORC1 inactivation, using 29 mutants deficient in genes encoding non-essential protein phosphatases. The ubiquitin-like protein Atg8 is recruited to the PAS and is required for the expansion of isolation membranes. We found that various protein phosphatases are required for efficient PAS formation of Atg8 and Atg1 after treatment of the TORC1 inhibitor rapamycin in

budding yeast. In particular, Pph22/PP2A, Pph3/PP4, and the dual-specificity protein phosphatase Yvh1 were critical for rapamycin-induced PAS formation of Atg8 and Atg1. Thus, PAS formation is regulated by protein phosphorylation/ dephosphorylation. Nevertheless, autophagy induction was not compromised in mutants under TORC1-inactive conditions. These findings suggest that PAS formation efficiency is not necessarily correlated with autophagic flux. In addition, there was no significant impact of the disruption of each phosphatase gene on TORC1 inactivation-mediated autophagy induction. It is likely that Atg13 dephosphorylation and autophagy induction after TORC1 inactivation are regulated by combination of multiple phosphatases.

Therefore, next we suspected that homologous Pph21 and Pph22 phosphatases (PP2A) are redundantly involved in autophagy regulation, because which is negatively regulated by TORC1. They become more active after TORC1 inactivation. Indeed, *pph21*Δ *pph22*Δ double deletion mutant (thereafter *pp2a*Δ) cells were defective in autophagy induction after rapamycin treatment and nutrient starvation. Autophagy induction after TORC1 inactivation by rapamycin treatment or nitrogen starvation was compromised in *pp2a*Δ cells, although autophagy induction was not inhibited in each single mutant, *pph21*Δ and *pph22*Δ. Additionally, Atg13 dephosphorylation was impaired in *pp2a*Δ cells. Consistently, *pp2a*Δ cells were defective in interaction of Atg13 with Atg17 and activation of Atg1 kinase. In addition, PAS formation was also repressed in *pp2a*Δ mutant. PP2A forms two different complexes with regulatory subunits, Cdc55 and Rts1 (referred as PP2A-Cdc55 and PP2A-Rts1), which have distinct functions. Interestingly, each single deletion of *CDC55* and *RTS1* failed to repress TORC1 inactivation-induced autophagy, whereas a double deletion of both genes impeded autophagy induction, like *pp2a*Δ. These findings demonstrate that PP2A-Cdc55 and PP2A-Rts1 cooperatively function in autophagy induction.

This study revealed that the orchestrated action of PP2A antagonizes Atg13 phosphorylation and promotes autophagy after the inactivation of TORC1 by switching “on” and “off” autophagy rapidly and effectively, and provides a good model toward autophagy regulation in human cells. Autophagy eliminates protein aggregates and damaged or superfluous organelles, which is important for maintaining cellular homeostasis. Autophagy deficiency causes various disease, Alzheimer’s, Parkinson’s, and Huntington’s disease, which are ameliorated by increase in autophagy activity. I hope that this study would contribute to progress of pharmacological treatments and human health.

Notice

1） The abstract should be about 800 words in English or 2,000 characters in Japanese.

2） Ample margins should be left, e.g., top, 30mm; bottom, 15mm; left, 30mm; right, 15mm.

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