

Public Abstract

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A UNIQUE WAY TO FORM A VESICLE: AMINOPEPTIDASE 1 AGGREGATION AND ITS BINDING TO RECEPTOR ATG19 FOR RECRUITMENT OF AUTOPHAGIC PROTEINS TO FORM A VESICLE IN THE CYTOPLASM-TO-VACUOLE-TARGETING PATHWAY IN YEAST

Misfolded protein aggregation causes disease and aging; autophagy counteracts this by eliminating damaged components, enabling cells to survive starvation. The Cytoplasm-to-vacuole-targeting (Cvt) pathway in yeast encompasses the aggregation of the premature form of Amino peptidase 1 (prApe1) in cytosol, its targeting by its autophagic receptor Atg19, and its sequestration inside a vesicle formed by autophagic proteins for vacuolar transport. The goal of this research was to elucidate the mechanism of prApe1 aggregation and binding to its receptor Atg19, to better understand how selective autophagy takes place and to develop an in vitro assay of autophagy. This study shows that the propeptide of Ape1 is important for aggregation and vesicle formation, it is sufficient for binding to prApe1 and Atg19. Defective aggregation disrupts vacuolar transport, suggesting aggregate shape is important in vesicle formation, while Atg19 and Atg11 binding is not sufficient for vacuolar transport. Ape1 dodecamerization may cluster propeptides into trimeric structures, with sufficient affinity to form propeptide hexamers by binding to other dodecamers, causing aggregation. Furthermore, the N- and C-terminal domains of Atg19 are critical for its correct localization on the surface of aggregates. Atg19 with N- or C-terminal deletions localizes inside aggregates instead, which could interfere with its binding to cytosolic autophagic proteins and cargo for vesicle formation. Meanwhile, the coiled-coil of Atg19 is sufficient for binding to the helix-turn-helix propeptide of prApe1. This study also shows that the mechanism of Atg19 binding to Ape1 aggregates is modified after starvation by the Atg1-Atg13 complex, which plays a key role in autophagy induction and pre-autophagosomal structure (PAS) assembly. A novel in vitro assay was developed, in which prApe1 aggregates and binds Atg19 and Atg8. This could be used as a scaffold for an in vitro assay of autophagosome formation to elucidate the mechanisms of autophagy.