Structural Basis for Receptor-Mediated Selective Autophagy of Aminopeptidase I Aggregates

Selective autophagy contributes to cellular homeostasis through degradation of various cargoes including protein aggregates and organelles. We reported structural and functional analysis of a selective cargo protein aminopeptidase I (Ape1) and its receptor Atg19. Ape1 forms a dodecamer, which is further tethered to others to form large aggregates via trimmer formation of the propeptide. Atg19 disassembles the propeptide trimer and forms a 2:1 heterotrimer with the propeptide, which not only blankets the Ape1 aggregates but also regulates their size. These receptor activities may promote elongation of the isolation membrane along the aggregate surface, thereby enabling sequestration of the cargo with high specificity.

Selective autophagy sequesters specific cargos including protein aggregates and various organelles within de novo generated autophagosomes, and translocates them to the lysosome for degradation. Selective autophagy regulates cellular homeostasis by degradation of unnecessary or harmful cargos and thus its disruption leads to severe diseases like cancer and neurodegenerative diseases [1]. Receptor proteins, specifically required for selective autophagy, recognize and recruit cargo proteins to the isolation membrane through the interaction with Atg8 that is conjugated to phosphatidylethanolamine in the isolation membrane [2]. To elucidate molecular mechanisms of selective autophagy, we focused on the Cvt pathway [3], which carries vacuolar hydrolases including aminopeptidase I (Ape1) by an autophagosome-like Cvt vesicle in budding yeast [4]. Ape1 itself is essential for the Cvt pathway since Ape1 functions as an essential template for Cvt vesicle formation by forming large aggregates as follows: after synthesis, a precursor form of Ape1 (prApe1) immediately self-assembles into a dodecamer, which further assembles into large aggregates termed the Ape1 complex via the function of the propeptide (residues 1-45 of prApe1). The propeptide also mediates the interaction with the receptor protein Atg19 [5, 6]. Atg19 recruits autophagy machineries such as Atg8 and a scaffold protein Atg11 to the Ape1 complex [7, 8], thereby initiating the elongation of the isolation membrane around the Ape1 complex. The interaction of Atg8 family proteins with receptor proteins including Atg19 has been well established [9, 10]. However, it remained to be elucidated how receptor proteins recognize a cargo. In this study, we studied the structures of Ape1 itself and its Atg19-bound form. We purified and crystallized prApe1 L11S mutant which is unable to be transported to the vacuole [5] because it was hard to purify wild type prApe1 due to aggregation. The crystal structure of prApe1 L11S forms a dodecamer (Fig. 1A) and is identical to M18 family proteases [11]. However, the electron density of most residues of the propeptide, which mediates Ape1 complex formation and Atg19 recognition, was disordered and not modeled. Thus, we then crystallized and determined the structures of Ape1 N22 (residues 1-22) alone and Ape1 N20-Atg19 coiled-coil (CC) at 3.4 and 1.9 Å resolution, respectively (Fig. 1B). Ape1 propeptide has an α-helical conformation and makes a trimer comprised of two parallel and one anti-parallel propeptides. The crystal structure of the Ape1 N20-Atg19 CC complex showed that Ape1 N20 and Atg19 CC also have an α-helical conformation and form a 1:2 heterotrimer, two parallel (Atg19 CC) and one anti-parallel (propeptide). Since the architecture is similar between propeptide trimer and propeptide-Atg19 CC heterotrimer, it was proposed that the homo- and hetero-trimer formation is exclusive to each other. In vitro assay revealed that the Ape1 propeptide oligomerization was actually inhibited by the addition of Atg19 CC. Furthermore, dynamic light scattering showed that the size of prApe1 aggregate (~1 μm) was reduced to ~100 nm upon addition of Atg19 CC. These results suggested that the assembly of Ape1 dodecamers through the formation of the propeptide trimer is competitively inhibited by Atg19 (Fig. 1C).

The other Cvt cargos, which are much smaller than the Ape1 aggregate, cannot function as a template for Cvt vesicle formation although they can bind Atg19 [4]. On the other hand, Ape1 P20L, which retains binding affinity to Atg19 and forms larger aggregates compared with wild-type Ape1, is not carried to the vacuole through the Cvt pathway [6–8]. We hypothesized that the proper size is important for the cargo to function as a template for Cvt vesicle formation since the size of the Cvt vesicle is uniformly ~150 nm, which is much smaller than autophagosomes, but is much larger than soluble proteins. To address this hypothesis, we tested whether cargo size affects Cvt vesicle biogenesis. We designed prApe1-Atg19 chimera protein that lacks CC. In Ape1 depleted cells, prApe1-Atg19 chimera formed aggregates that were too large to be transported to the vacuole. However, co-expression of Atg19 CC reduced the aggregation size and the chimera protein was successfully transported to the vacuole through the Cvt pathway. These results indicated that the role of Atg19 is not only linking cargo to the isolation membrane, which is generally considered to be the main role of receptor proteins, but also regulating the cargo size optimal for selective autophagy (Fig. 2). Further studies are required to clarify whether this new function of Atg19, that is, regulating the cargo size, is a common feature among various autophagy receptors.

REFERENCES

Figure 1: Structural studies of Ape1 and Ape1-Atg19 complex. (A) Crystal structure of prApe1 L11S. One protomer is colored yellow, in which the propeptide portion is colored red. (B) Crystal structure of propeptide homotrimer (left) and propeptide-Atg19 heterotrimer (right). (C) Proposed model of Ape1 self-oligomerization and competitive recognition by Atg19.

Figure 2: Proposed mechanism of Atg19-mediated selective autophagy of Ape1 aggregates.