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Structure of complexes between ATG5 and its binding partners for autophagosome maturation

Jun Hoe Kim and Hyun Kyu Song*
Division of Life Sciences, Korea University
145 Anam-ro, Seongbuk-gu, Seoul 136-713, South Korea

1 Introduction

Autophagy is a tightly regulated lysosome-mediated catabolic process in eukaryotes that maintains cellular homeostasis. A distinguishable feature of autophagy is the formation of double-membrane structures, autophagosome, which envelopes the intracellular cargoes and finally degrades them by fusion with lysosomes. So far, many structures of Atg proteins working on the autophagosome formation have been reported, however those involved in autophagosome maturation, a fusion with lysosome, are relatively unknown. One of the molecules in autophagosome maturation, TECPR1, has been identified and recently, structural studies on both ATG5-TECPR1 and ATG5-ATG16L1 complexes revealed that TECPR1 and ATG16L1 share the same binding site on ATG5. We reported these results in combination with supporting biochemical and cellular biological data providing an insight into a model for swapping ATG5 partners for autophagosome maturation [1,2].

2 Experiment

Human His-tagged ATG5 was co-expressed with N-terminal 69 residues of ATG16L1 (ATG16N69) or AIR of TECPR1 (TECAIR) using pETDuet-1 vector. The over-expressed proteins were co-purified by nickel-NTA affinity chromatography followed by anion exchange and size exclusion chromatography. ATG5-ATG16N69 was crystallized using the hanging drop method by vapor-diffusion at 20 ℃. In case of ATG5-TECAIR, initial twinned crystals were improved by the micro-seeding technique and growth at 4 ℃. ATG5-ATG16N69 crystal diffracted up to 2.7 Å and the number of molecules in asymmetric unit was 3. The space group and unit cell parameters were $P4_12_12$ with a=b=93.09 Å and c=245.6 Å. ATG5-TECAIR crystal diffracted up to 1.8 Å with 1 molecule per asymmetric unit and belongs to $P2_12_12_1$ space group with a=43.62 Å, b=71.92 Å and c=96.35 Å. Diffraction data were collected using an ADSC quantum CCD detector at the NW12 beamline of Photon Factory. 180-200 images were collected with 1° oscillation, and each image was exposed for 3-5 seconds. The diffraction data were processed and scaled using the HKL2000 software package. Phases were obtained by molecular replacement with previously determined yeast Atg5 structure [3] using MOLREP in the CCP4 program suite. The initial model was manually built and refined using COOT and PHENIX.

3 Results

Human ATG5 consists of 2 ubiquitin-fold domains which are the equivalent of the ubiquitin-like domains of yeast Atg5 and has a similar binding mode on ATG16N69 and TECAIR (Fig. 1A). According to a sequence alignment, it was revealed that several residues on the helix of ATG16L1 and TECPR1 are conserved among species. We identified the motif as W-X_3-I-X_2-L-X_3-Q/E and named as ATG5-interacting motif (AFIM). Biochemical and cell biological data supported the significance of the motif [2] and finally we suggested a model of autophagosome maturation mechanism by swapping of ATG5 interaction partners (Fig. 1B).

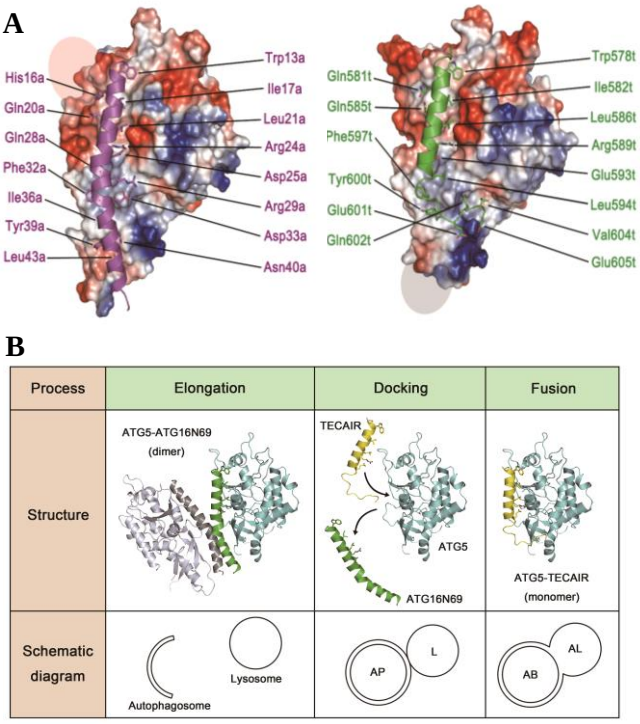

Fig. 1 (A) Overall structures of ATG5-ATG16N69 (left) and ATG5-TECAIR (right). (B) A simplified model for autophagosome maturation mechanism by swapping ATG5 binding partners.

References

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[3] Matsushita, M. *et al*. *JBC* 282: 6763-6772 (2007)

*hksong@korea.ac.kr