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Crystal structure of plant Atg7-Atg3 complex

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1 Introduction

Autophagy is an intracellular degradation process conserved from yeast to higher eukaryotes such as plants and mammals. Atg8 is initially activated by Atg7 (an E1 like enzyme) and is then transferred to Atg3 (an E2 like enzyme), and is finally conjugated to phosphatidylethanolamine (PE). On the other hand, Atg12 is initially activated by the same E1 enzyme, Atg7, but is transferred to another E2, Atg10, rather than Atg3, and is finally conjugated to Atg5. It is not understood how Atg7 recognizes two distinct E2s and transfers Atg8 to Atg3 and Atg12 to Atg10 in a proper manner.

2 Experiment

The N-terminal domain (NTD) of *Arabidopsis thaliana* (At) Atg7 and AtAtg3 expressed in *E. coli* were purified by affinity chromatography by using a glutathione-Sepharose 4B column, followed by excision of GST with HRV 3C protease. They were further purified by tandem chromatography by using Superdex 200pg gel filtration, glutathione-Sepharose 4B and Resource Q anion-exchange columns. The crystallization sample of the AtAtg7^{NTD}–AtAtg3 complex was at the concentration of 12.3 mg/ml (1:1 molar ratio) in 20 mM Tris-HCl, pH 8.0, 2 mM DTT, ~150 mM NaCl, and co-crystallized as a complex at 293 K by the sitting-drop vapor-diffusion technique using polyethylene glycol 3350 as a precipitant. For preparation of selenomethionine (SeMet)-labeled crystals, only AtAtg3 was labeled with SeMet, and the AtAtg7^{NTD} (native)–AtAtg3 (SeMet) complex was used for crystallization. Crystals were cryoprotected through addition of 15% ethylene glycol (v/v), then flash cooled and kept in a stream of nitrogen gas at 100 K during data collection. The crystal structure of AtAtg7^{NTD}–AtAtg3 was solved by single-wavelength anomalous dispersion (SAD) phasing by using a crystal of the SeMet derivative. SAD phasing, density modification and initial model building were performed with the automated software suite PHENIX. Model building and modification were performed manually with the molecular modeling program COOT, followed by iterative rounds of refinement by using the REFMAC5 in the CCP4 software suite

3 Results and Discussion

The obtained crystal ($P222_1$, a = 101.52, b = 132.68, c = 102.81 Å) diffracted X-rays to 3.1Å resolution. The structure has been determined by the single-wavelength anomalous dispersion method using a selenomethionine-substituted crystal. AtAtg7^{NTD} and AtAtg3 form a 1:1 heterodimer in the crystal. The AtAtg7^{NTD}-AtAtg3 complex structure reveals not only the similarity and difference of Atg3 and Atg10 recognition by Atg7 [1], but also the variable conformation of the catalytic site of Atg3 [2], which will be a basis for elucidating the molecular mechanisms of Atg8 conjugation.

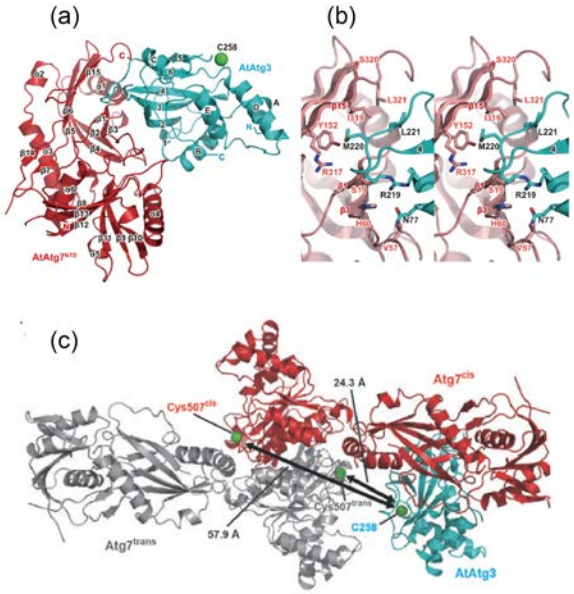

Fig. 1: Structure of AtAtg7-AtAtg3 complex. (a) Overall structure of AtAtg7^{NTD} –AtAtg3 complex. AtAtg7^{NTD} and AtAtg3 are colored *red* and *cyan*, respectively. Sulfur atom of the catalytic cysteine residue (Cys258) of AtAtg3 is shown with a *green* sphere model. (b) Close-up stereo view of AtAtg7^{NTD} – AtAtg3 complex. AtAtg7^{NTD} and AtAtg3 are colored salmon pink and cyan, respectively. (c) Modeled structure of AtAtg3 bound to full-length Atg7. AtAtg3, Atg7^{cis} and Atg7^{trans} are colored *cyan*, *red* and *gray*, respectively. Sulfur atoms of the catalytic cysteine residues are shown with a *green* sphere model. Arrows indicate the distance between the catalytic cysteine residues of E1 and E2.

References

[1] Yamaguchi *et al*., *Nat. Struct. Mol. Biol.* **19**, 1250-6 (2012).

[2] Sakoh-Nakatogawa et al., *Nat Struct. Mol. Biol*. **20**, 433-9 (2013)

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