

## Crystal structure of Atg7 in complex with Atg8

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### Introduction

Autophagosome formation is a very essential event during macroautophagic process for disassembling and recycling cellular components via lysosome in response to various signals. Two ubiquitin-like molecules, Atg12 and Atg8 play crucial roles for creation of this double-membrane vesicle sharing a common E1-like enzyme, Atg7. The N-terminal domain of Atg7 (Atg7N) reveals a unique protein fold and interacts with both autophagic E2-enzymes, Atg10 and Atg3. The C-terminal domain of Atg7 (Atg7C) in complex with Atg8 shows the mode of dimerization and mechanism of recognition of Atg8. Notably, the catalytic cysteine residue in Atg7 is positioned close to the C-terminal glycine of Atg8, its target for thioester formation, potentially eliminating the need for large conformational rearrangements characteristic of other E1s.[1]

### Methods

Purified Atg7C (C507S mutant) and Atg8 were mixed in a molar ratio of 1:2 at 4 °C for 30 min and reloaded onto a gel-filtration column for purification of the complex. Samples were concentrated to approximately 10~14 mg/ml and crystallized by hanging drop vapor diffusion at 22 °C, after mixing of an equal volume of reservoir solution containing 0.1 M HEPES (pH 7.5), 7~10% (w/v) PEG3,350 and 20 mM proline. Atg7N was concentrated to 16 mg/ml, and crystals were also obtained by hanging-drop vapor diffusion at 22 °C with a reservoir solution containing 0.1 M Na-HEPES (pH 8.0), 1.5M Li<sub>2</sub>SO<sub>4</sub> and 0.1M spermidine tetrahydrochloride. Crystals were flash-frozen with reservoir solution containing 20% (v/v) glycerol in a nitrogen stream at 100 K. All of the eight possible selenium sites in the asymmetric unit of Atg7C–Atg8 crystal, and two of three possible sites in Atg7N, were located using three-wavelength MAD data sets. Initial phases were calculated, and initial models for Atg7C–Atg8 and Atg7N were built automatically using the PHENIX package program with up to 90% of Atg7C–Atg8 and only 20% of the Atg7N polypeptide chain. Models were rebuilt manually using COOT and O. Refinement was also carried out using the PHENIX package program.

### Results

The crystal structure of Atg7C–Atg8 complex reveals a compact dimer with approximate dimensions of 85 Å × 72 Å × 50 Å (Fig 1a). Each subunit (residues Asp294–Glu620) consists of 11 α-helices, four

3<sub>10</sub>-helices, eight β-strands and connecting loops, and includes the adenylation region, a zinc-binding site and a catalytic cysteine residue (Cys507) for thioester bond formation. The binding surface for Atg8 can be divided into two parts: one region for binding the core of the domain and another for recognition of its C-terminal tail, including Gly116, the target of adenylation. The C-terminal helices of Atg7, in particular α17, are crucial for coordination of the core of Atg8. Primarily hydrophobic residues form the binding interface between Atg7C and Atg8, with some contribution from polar residues and hydrogen bonds. The buried surface area of the Atg7C–Atg8 complex is approximately 2,300 Å<sup>2</sup>.

The isolated Atg7N covering 1–294 amino acids is monomeric and has an elongated shape with approximate dimensions of 64 Å × 38 Å × 34 Å (Fig 1b). It consists of six α-helices, three 3<sub>10</sub>-helices, 16 β-strands and connecting loops organized in two structural subdomains. Using a SEC-MALS assay, we revealed that Atg7N forms 1:1 complex with E2s (Atg3 and Atg10). Therefore, Atg7N is defined as an ‘autophagic E2-binding domain’.

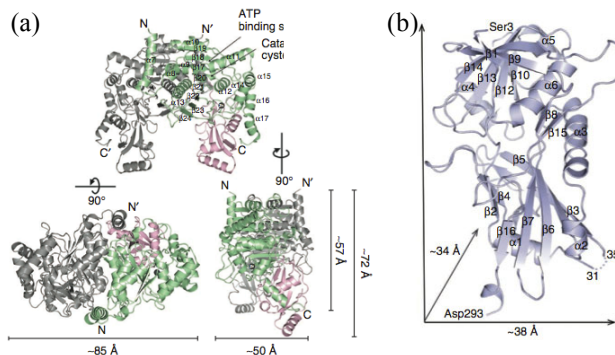


Figure 1. Structure of yeast Atg7. (a) Ribbon diagram of the structure of the Atg7C–Atg8 complex showing 2:2 stoichiometry. In one Atg7C–Atg8 pair, Atg7C is colored green and Atg8 is pink; the other pair is colored gray for clarity. (b) Ribbon diagram showing the structure of Atg7N (blue). The secondary structural elements are labeled and the N- and C-terminal residues of Atg7N are indicated. The region absent in the electron density map (residues 32–34) is indicated as a dotted line.

### Reference

[1] S.B. Hong et al., Nature Struct. Mol. Biol. 18, 1323 (2011)

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