

Three-Dimensional Structure of *Arabidopsis thaliana* ATG9

Louis Tung Faat Lai, Chuanyang Yu, Jan Siu Kei Wong, Ho Sing Lo, Samir Benlekbir, Liwen Jiang, Wilson Chun Yu Lau
The Chinese University of Hong Kong, Hong Kong SAR

Macroautophagy/autophagy is an essential process for the maintenance of cellular homeostasis by recycling macromolecules under normal and stress conditions. ATG9 (autophagy related 9) is the only integral membrane protein in the autophagy core machinery and has a central role in mediating autophagosome formation. In cells, ATG9 exists on mobile vesicles that traffic to the growing phagophore, providing an essential membrane source for the formation of autophagosomes. Here we report the three-dimensional structure of ATG9 from *Arabidopsis thaliana* at 7.8 Å resolution, determined by single particle cryo-electron microscopy. ATG9 organizes into a homotrimer, with each protomer contributing at least six transmembrane α -helices. At the center of the trimer, the protomers interact via their membrane-embedded and C-terminal cytoplasmic regions. Combined with prediction of protein contacts using sequence co-evolutionary information, the structure provides molecular insights into the ATG9 architecture and testable hypotheses for the molecular mechanism of autophagy progression regulated by ATG9.

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