BL-1A, BL-17A/ 2018RP-12

# Structural study on Atg2 that mediates autophagosomal membrane expansion

Takuo OSAWA[1] and Nobuo N. NODA[1,*]
[1] Institute of Microbial Chemistry, Microbial Chemistry Research Foundation,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

## 1 Introduction

Autophagy is an intracellular degradation system conserved among eukaryotes. When autophagy is induced, a membrane structure called an isolation membrane (IM) suddenly appears in the cytoplasm, which expands and seals into a double membrane organelle, an autophagosome, where a portion of the cytoplasm is sequestered. Autophagosome then fuses with the lysosome or vacuole and the inner contents are degraded by lysosomal hydrolases [1]. For autophagosome formation, IMs must receive phospholipids from membrane-source organelles, such as ER, as building blocks. However, the mechanism of how phospholipids are transported to the IM through aqueous cytoplasm remained to be elucidated.

Atg2 is one of the core Atg proteins that mediate autophagosome formation and localizes to the expanding edge of the IM [2]. Since Atg2 also contacts with the ER [2], it was proposed that Atg2 functions as a tether between the ER and the IM. However, the molecular functions of Atg2 have been unknown due to its low sequence homology with characterized proteins. Overall structure of human Atg2 was reported by negative-staining electron microscopy at low resolution, which showed a club-like architecture [3, 4]. Here, in order to obtain high-resolution structural data on Atg2, we crystallized the N-terminal region (NR) of Atg2 and determined the crystal structure [5].

## 2 Experiment

S. pombe Atg2$^{NR}$ was expressed in E. coli as a fusion protein with a T4 lysozyme and a His-tag. After affinity purification using a TALON resin column, the protein was subjected to size-exclusion chromatography using a Superdex 200 column. For preparation of PE-bound form, fusion protein was mixed with PE in a 1:2 molar ratio for 1 h before size-exclusion chromatography. Purified fusion protein was crystallized by the hanging drop vapour diffusion method using 15-16% PEG400, 0.1 M Bis-Tris (pH 6.5), and 0.2 M ammonium sulfate as a reservoir solution. The crystals were soaked in the reservoir solution containing 30% PEG400 and 2% trehalose as a cryoprotectant. X-ray diffraction data were collected at BL-1A and BL-17A of the Photon Factory, and processed using HKL2000. Initial phasing was performed by a combination of the molecular replacement and single wavelength anomalous dispersion methods using AutoMR and AutoSol in Phenix. Models were built manually with COOT and crystallographic refinement was performed with Phenix.

## 3 Results and Discussion

Data collection and refinement statistics are summarized in Table 1. Atg2$^{NR}$ is comprised of a twisted β-sheet and a helical region, which together assume a globular structure with a large hydrophobic cavity (Fig. 1). The structure of the Atg2$^{NR}$-PE complex reveals that the hydrophobic cavity accommodates the acyl portions of the PE. Since this interaction mode is similar to other lipid transfer proteins, we studied the lipid transfer activity of Atg2 in vitro and revealed that Atg2 actually possesses lipid transfer activity between liposomes. Moreover, from mutational studies of Atg2 both in vitro and in vivo, we proposed a model that Atg2 tethers the ER to the IM and at the same time mediates phospholipid transfer from the ER to the IM for expanding the IM into the autophagosome [5, 6].

Table 1: Data collection and refinement statistics

| | T4L-SpAtg2$^{NR}$ (PDB 6A9E) | PE-bound form (PDB 6A9J) |
|---|---|---|
| **Data collection** | | |
| Space group | $P2_1$ | $P2_1$ |
| Cell dimensions | | |
| *a*, *b*, *c* (Å) | 86.5, 62.7, 86.3 | 86.7, 62.9, 86.7 |
| $\alpha$, $\beta$, $\gamma$ (°) | 90.0, 91.8, 90.0 | 90.0, 91.9, 90.0 |
| | *Peak* | |
| Wavelength | 0.9692 | |
| Resolution (Å)[a] | 50-3.20 (3.26-3.20)[a] | 50-2.70 (2.75-2.70) |
| $R_{merge}$ | 0.145 (0.579) | 0.381 (0.439) |
| $I/\sigma(I)$ | 16.8 (3.4) | 23.8 (2.6) |
| Completeness (%) | 100 (100) | 99.9 (99.4) |
| Redundancy | 13.7 (14.3) | 6.6 (6.2) |
| **Refinement** | | |
| Resolution (Å) | 44-3.2 | 38-2.7 |
| No. reflections | 29590 | 25884 |
| $R_{work}$ / $R_{free}$ | 0.224/0.266 | 0.251/0.268 |
| No. atoms | | |
| Protein | 5023 | 4998 |
| PE | | 102 |
| *B* factors | | |
| Protein | 63.0 | 67.8 |
| PE | | 68.2 |
| R.m.s deviations | | |
| Bond lengths (Å) | 0.003 | 0.003 |
| Bond angles (°) | 0.644 | 0.863 |

[a]Values in parentheses are for highest-resolution shell.

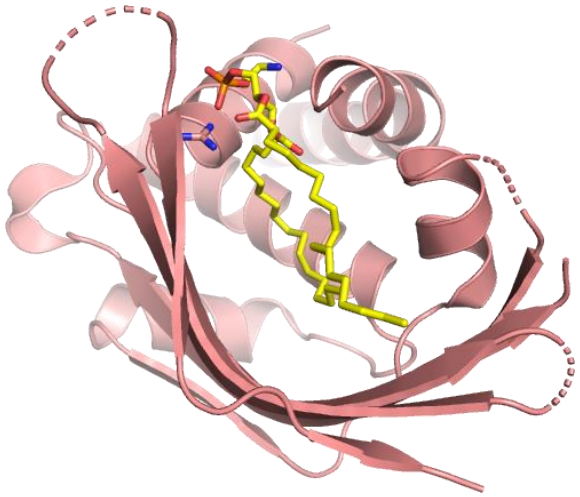

Fig. 1: Structure of Atg2$^{NR}$ complexed with PE.

Acknowledgement

We thank Y. Ishii for her help with protein expression. S. pombe Atg2 cDNA was provided by the RIKEN BRC through the National BioResource Project of the MEXT/AMED, Japan. This work was supported by Japan Science and Technology Agency (JST) CREST and the Japan Society for the Promotion of Science (JSPS) KAKENHI.

References

[1] Noda, N. N. and Inagaki, F. *Annu Rev Biophys* **44**, 101 (2015).
[2] Suzuki, K. et al. *J. Cell Sci.* **126**, 2534 (2013).
[3] Chowdhury, S. et al. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E9792 (2018).
[4] Zheng, J. X. et al. *Autophagy* **13**, 1870 (2017).
[5] Osawa, T. et al. *Nat. Struct. Mol. Biol.* **26**, 281 (2019).
[6] Osawa, T. and Noda, N. N. *Protein Sci.* **28**, 1005 (2019).

* nn@bikaken.or.jp