BL-1A, BL-17A/2015G703, 2016G170

Crystal structure of hetero-trimeric core of LUBAC, a unique ubiquitin ligase for linear ubiquitination

Akira Tokunaga*[1], Hiroaki Fujita[2], Erik Walinda[2], Izuru Ohki[1], Mariko Ariyoshi[3], Kazuhiro Iwai[2], Hidehito Tochio[4], and Masahiro Shirakawa[1]

[1]Department of Molecular Engineering, Graduate School of Engineering, Kyoto University, Kyoto 615-8510, Japan
[2]Department of Molecular and Cellular Physiology, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan
[3]Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka 565-0871, Japan
[4]Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

1 Introduction

Linear ubiquitin chain assembly complex (LUBAC) is a unique ligase synthesizing a linear ubiquitin chain, in which the amino group of the N-terminal Met of one ubiquitin and the C-terminal carboxyl group of another are conjugated. In nuclear factor-kappa B (NF-κB) signaling pathway, LUBAC attaches the linear ubiquitin to NF-κB essential modulator (NEMO) and promotes transcription of genes encoding several cytokines, chemokines, or cell adhesion molecules, activating the immune response or cell death repression. LUBAC is composed of the catalytic subunit HOIP and the accessory subunits HOIL-1L and SHARPIN. It has been reported that mice lacking SHARPIN suffer from auto-inflammation and immunodeficiency, most likely due to destabilization of LUBAC and consequential dysregulation of NF-κB signaling. Although this implies that the integrity of the three subunits is needed for the catalytic activity of LUBAC, the structural details of simultaneous trimeric interactions remain elusive.

Here we report a crystal structure of the core region of LUBAC at 2.40 Å resolution, which explains the ternary intermolecular interactions essential to functional LUBAC formation [1].

2 Experiment

Single-wavelength (0.98Å) data for the core region of LUBAC was collected on the beamline 1A at Photon Factory. The data was processed using HKL2000 and the CCP4 program suite.

3 Results and Discussion

The structure of the trimeric core of LUBAC was determined using molecular replacement and refined at a resolution of 2.40 Å to a crystallographic *R*-factor of 24.4%. The final model was comprised of amino acid residues 2-136 (A chain; HOIL-1L), 473-629 (B chain; HOIP), 169-300 (C chain; SHARPIN), a glycerol molecule, and 21 waters (Fig).

The three subunits form a trimeric complex with a 1:1:1 stoichiometry. HOIP constitutes the ubiquitin-associated (UBA) region comprising seven α helices and two 3_{10} helices, in which two UBA-like modules are tandemly linked via a middle α4 helix. HOIL-1L and SHARPIN share the serial motifs of a LUBAC tethering motif (LTM) and a ubiquitin-like (UBL) domain. HOIP UBA binds the UBL domains of HOIL-1L and SHARPIN mainly using its N- and C-terminal UBA modules, respectively, though the binding modes are completely different from each other. In addition, the structure demonstrates a novel interaction between the LTMs of the HOIL-1L and SHARPIN, in which the helix-helix-strand motifs of LTMs are swapped to fold into a domain-like architecture, denominated as the tethering domain. The structural basis of LUBAC

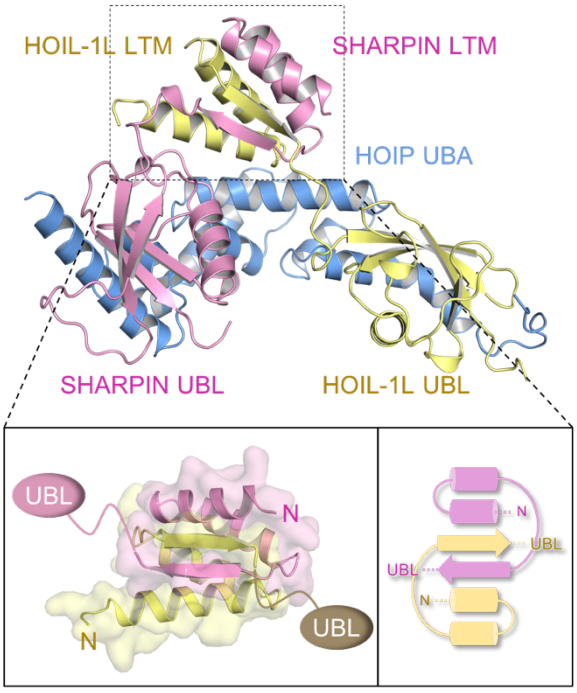

Fig: (Top) Overall structure of the core region of LUBAC. The structure of HOIP UBA, SHARPIN LTM-UBL, and HOIL-1L LTM-UBL are shown in blue, pink, and yellow, respectively. (Bottom) The structure of the tethering domain is shown in the left panel. The right panel displays a topology diagram of the tethering domain.

formation established by the findings of the simultaneous trimeric interactions would be valuable not only for understanding the molecular mechanism of LUBAC destabilization and dysregulation, but also for targeting LUBAC in therapeutics.

References

[1] Fujita, H., Tokunaga, A., *et al.* (2018) *Cell Rep.* **23**(4), 1192–1204.

* tokunaga.akira.38w@st.kyoto-u.ac.jp