The Ubiquitin System: Structural insights into the formation of linear ubiquitin chains

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Abstract

The ubiquitin system is a network of proteins dedicated to regulating nearly every cellular process. Ubiquitin is a highly conserved protein which is attached to target proteins often in the form of chains of many ubiquitin molecules through a process called ubiquitination. Ubiquitination modifications are dynamic and versatile and the physiological response generated depends on the intermolecular connection between the ubiquitin molecules. Misregulation of the ubiquitin system has been implicated in the development of many diseases such as cancer, neurodegenerative and immune diseases.

Linear (M1-linked) ubiquitin chains have emerged recently as important regulators of immune and inflammatory pathways. This type of chains is exclusively synthesized by an E3 ligase termed LUBAC (linear ubiquitin chain assembly complex). In this study, we used X-ray crystallography to visualise the three-dimensional structure of an ubiquitin protein bound to the catalytic site of LUBAC. The structure allowed us to perform biochemical analysis and obtain important insights into how enzymes are able to generate specific types of ubiquitin chains.

These findings contribute to a better understanding of the ubiquitin system and may consequently lead to the development of new treatments for a number of diseases.

Figure 1: Structure and function of ubiquitin chains

Figure 2: Linear ubiquitin chains mediate activation of NF-kB

Figure 3: The ubiquitination cascade

The process of ubiquitination is performed by an enzymatic cascade consisting of three enzymes: ubiquitin-activating enzyme (E1), ubiquitin conjugating enzyme (E2) and ubiquitin ligase (E3). The C-terminal of ubiquitin is first attached in an ATP-dependent reaction to the active cysteine residue in the E1 system through a thioester bond. Subsequently, it is transferred to the active site cysteine in the E2 system through a thioester bond. Finally, ubiquitin is transferred from the E2 onto the lysine side-chain of the substrate protein or the catalytic cysteine residue in the E1 enzyme through a thioester bond. Subsequently, it is dissociated from the NF-κB and the unique LDD extension. EMBO J. 31, 3833-3844 (2012).

Figure 4: Domain architecture of the LUBAC

LUBAC (linear ubiquitin chain assembly complex) is a complex composed of three proteins: HOIL-1L, HOIP and SHARPIN. LUBAC synthesizes linear ubiquitin chains to the regulatory subunit (NEMO or IKKβ) of the IKK complex (composed of IKKα, IKKβ and NEMO) which promotes phosphorylation and activation of the IKK complex. Activated IKK, in turn, phosphorylates and activates the transcription factor NF-κB. In contrast to RBR-C, which lacks the N-terminal and the unique LDD extension, SHARPIN displays the characteristic structure of RING and HECT ligases and is represented by the RBR (RING between RING) family of E3s.

Figure 5: The RBR-C of HOIP synthesizes linear ubiquitin chains via a thioester intermediate

Fluorescently labelled ubiquitin (Cy5-ubiquitin) allows the detection of all the ubiquitin-thioester conjugates which are formed in a reconstituted ubiquitination cascade, in vitro. Simple loading on standard SDS-PAGE and subsequent fluorescence scanning reveals that thioester intermediates are not only formed between ubiquitin and E1 and E2 enzymes but also between ubiquitin and thioester. The RBR-C construct which includes the RBR domain and the C-terminal extension (referred to as RBR-C) lyses ubiquitin E1 in the absence of ATP but not in the presence of ATP and the C-terminal extension. Lysine Ubiquitin is transferred from the E2 onto the lysine side-chain of the substrate protein or the catalytic cysteine residue in the E1 enzyme through a thioester bond. Subsequently, it is dissociated from the NF-κB and the unique LDD extension. EMBO J. 31, 3833-3844 (2012).

Figure 6: CBR-C is the minimal catalytic core of HOIP

In vitro ubiquitination assays using the RBR-C and the RING2-C construct of HOIP which is referred as CBR-C (CBR-C) allowed the analysis of the mass transfer by SDS-PAGE. The RBB-C construct which lacks RING1 and RING2 domains which were suspected to be required for catalysis is still able to generate ubiquitin chains albeit at a slower rate when compared to RBR-C. To confirm that CBR-C had restored the ability to specifically synthesize linear ubiquitin chains, a ubiquitination assay was performed with ubiquitin that was conjugated to an N-terminal His tag and was no longer able to produce linear chains.

Figure 7: Crystal structure of the catalytic core of HOIP

The protein crystals obtained for HOIP CBB-C were taken to Diamond Synchrotron (Oxfordshire), targeted with an X-ray beam which diffracted by the crystal to high resolution and produced a diffraction pattern. The two-dimensional images obtained were converted into a three-dimensional model of the density of electron within the crystal using mathematical methods and computer software. The crystal structure of HOIP CBB-C revealed a novel superfold, which can be divided into four subdomains: the CBB (catalytic BB), β-hairpin and two NZ domains which are tightly interconnected with a helical base.

Figure 8: Snapshot of the linear ubiquitin chain synthesis catalyzed by HOIP

The crystal structure of HOIP CBB-C in complex with acceptor (orange) and donor (yellow) ubiquitin is presented. Left: The positions of the catalytic CBB of HOIP to the G76 of donor ubiquitin and the MI of acceptor ubiquitin are indicated. Right: The HOIP CBB-C–ubiquitin complex with HOIP CBB-C shown in a surface representation to emphasize the spatial relationship between the three molecules.

Summary

- The RBB of HOIP forms a thioester intermediate with ubiquitin, before transferring it to a substrate supporting a general RING/HECT hybrid mechanism.
- The cysteine-carrying RING2 plus a C-terminal extension (HECT) HOIP is the minimal catalytic core of HOIP required to form linear ubiquitin chains.
- The structure of HOIP CBB-C revealed a novel fold which provides the platform for the formation of linear ubiquitin chains.
- The structure of HOIP CBB-C is complex with donor and acceptor ubiquitin provided an unique snapshot of the conjugation of linear ubiquitin chains by HOIP.

References