HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 4 (2024)

The Ubiquitination Process

Minseo Cho[1] and Gang Xie[#]

[1]Eastern Christian High School, USA
[#]Advisor

ABSTRACT

Ubiquitination is an important post-translational modification that modulates many biological processes, including protein degradation, immune responses, and protein localization. During the process of ubiquitination, proteins are covalently modified with a single ubiquitin and poly-ubiquitin chains through a specific isopeptide bond. The ubiquitination process has three main steps: ubiquitin activation, ubiquitin conjugation, and ubiquitin ligation, which are catalyzed by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3), respectively. Here the chemistry and the biochemical mechanism of the ubiquitination process is discussed.

Introduction

Ubiquitin (Ub) is a small (8.6 kDa) regulatory protein that consists of 76 amino acids, containing 7 lysine residues (Lys 06, Lys 11, Lys 27, Lys 29, Lys 33, Lys 48, Lys 63). Ubiquitin has two terminals, N-terminal Met 01 residue and C-terminal G76 residue (NH^{3+} and COO^{-} at isoelectric point, respectively). It is found ubiquitously in most tissues of eukaryotic organisms. Ubiquitin regulates virtually all aspects of eukaryotic biology. All eukaryotes (from yeast to humans) express the enzymatic machinery to covalently modify substrate proteins with ubiquitin in a process termed as ubiquitination (Damgaard 2021). Ubiquitination is a post-translational modification that modulates several biological processes, including protein degradation, immune responses, apoptosis, and protein localization. During the process of ubiquitination, proteins are modified with poly-ubiquitin chains in which the linkage between ubiquitin molecules encodes information about the substrate's fate in the cell.

Ubiquitylation involves three main steps: activation, conjugation, and ligation, performed by ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s), respectively. The main result of this sequential cascade is to attach ubiquitin covalently to lysine residues on the protein substrate via an iso-peptide bond. In addition, ubiquitin can also be bound to cysteine residues of protein substrate through a thioester bond, serine and threonine residues through an ester bond, or the amino group of the protein's N-terminus via a peptide bond (Stewart et al., 2016).

Ubiquitin modifications control many vital cellular processes through proteolytic and non proteolytic mechanisms, including proteasomal degradation and proteostasis, selective autophagy, cell signaling cascades such as those for NF-κB activation, protein trafficking, DNA repair and genome integrity, cell cycle control, and programmed cell death (Damgaard 2021).

Ubiquitin Activation

E1 Domains

E1 enzymes undergo large-scale structural changes within their distinct domains, and such rearrangements allow E1 enzymes to catalyze multistep reactions. E1 enzymes have two Rosmann-type subunits, inactive and active Rosmann-type subunits, and two domains–the cysteine domain and the ubiquitin-fold domain (UFD). The active Rosmann-type

is responsible for catalyzing adenylation (Lorenz et al., 2013). The cysteine domain is split into two domains, first and second catalytic cysteine half domains, FCCH and SCCH, respectively. The FCCH domain plays a role in Ub recognition, and the SCCH domain contributes to harboring the catalytic cysteine residue involved in thioesterification of Ub (Yuan et al., 2021).

Conformational Changes in E1 Domains during Ubiquitin Activation

Ubiquitin activation involves two steps catalyzed by E1: Adenylation of Ub and thioesterification reaction of Ub. In adenylation, E1 binds both ATP and Ub to liberate pyrophosphate(PPi) from ATP. During adenylation, the SCCH domain of E1 removes catalytic cysteine from the adenylation site in the active Rossmann-type subunit, causing an open conformation of the cysteine domain (Lorenz et al., 2013 & Yuan et al., 2021). In this open state, Ub is recognized by the Rosmann-type subunit through hydrophobic contacts. Then, the carboxy-terminal flexible tail of Ub extends towards the ATP binding pocket in the active Rossmann-type subunit (Lorenz et al., 2013). During adenylation, E1 is reported to be Mg^{2+} dependent since an Mg^{2+} neutralizes the charge of ATP, stabilizes the transition state, and neutralizes PPi. In the Rossmann-type subunit, the negatively charged carboxylate group (C-terminus) of ubiquitin nucleophilically attacks an electrophilic alpha phosphate group of ATP, and PPi is liberated. As a result, an ubiquitin-adenylate (Ub~AMP) intermediate is formed, and the intermediate serves as the donor of ubiquitin to a cysteine in the E1 active site. Prior to the adenylation, the negatively charged carboxylate group of ubiquitin C-terminus is a bad leaving group. However, after creating a good leaving group during the adenylation, the Ub~AMP becomes reactive (Schmelz and Naismith 2009).

The cysteine residue of E1 nucleophilically attacks the electrophilic carbon of the C-terminus of ubiquitin (thioesterification) and releases the AMP. During thioesterification, the SCCH domain undergoes a ~125 degree rotation, leading to the closed conformation of the cysteine domain, in which the SCCH domain forms extensive contacts with the active Rossmann-type subunit, thus allowing the nucleophilic attack of cysteine residue towards the ubiquitin C-terminus (Lorenz et al., 2013 & Yuan et al., 2021). After thioesterification, the cysteine domain adopts the open state once again for adenylation of a second Ub, causing E1 to be loaded with two Ub proteins, one that is thioester-linked to the catalytic cysteine of the SCCH domain and a second one non-covalently bound in the adenylation site of the active Rossmann-type subunit (Lorenz et al., 2013 & Yuan et al., 2021).

Ubiquitin Conjugation

During Ub conjugation, where E2 enzymes carry activated ubiquitin to their active site cysteine group (transthioesterification), E1 enzymes adopt large-scale structural changes within their distinct domains. In contrast, most E2 enzymes are relatively small, have a singular UBC domain, and do not undergo striking conformational changes (Lorenz et al., 2013 & Stewart et al., 2016). During trans-thioesterification, E2 enzyme is recognized in tripartite manner by the doubly loaded E1 enzyme: one set of interactions is provided by UFD domain of E1, another is contributed by the active Rosmann-type subunit, and the last one is promoted by the Ub that is thioester-linked to the catalytic cysteine (Lorenz et al., 2013). During the adenylation of the second Ub in the active Rosmann-type subunit, SCCH domain that contains the thioester linked Ub adopts the open state. UFD domain accommodates this re-oriented thioester-linked Ub in the double loaded E1 by adopting a distal conformation, leading to a distance of ~25 Å between the E1 and E2 active sites, resulting in the recognition and recruitment of E2 enzyme (Lorenz et al., 2013 & Yuan et al., 2021). After the recruitment of E2 enzyme, UFD domain of E1 undergoes a transition from the distal to the proximal conformation, which triggers the active site of E2 to be closer to that of E1, leading to tran-thioesterification (Yuan et al., 2021).

Ubiquitin Ligation

Types of E3

During ligation, E3 enzyme transfers the ubiquitin from E2 to the substrate, creating an isopeptide bond between the carboxyl terminus of ubiquitin and a primary amino group of the target protein. There are three types of E3 that are used in the transfer of Ub from E2 to the target protein: RING-type E3, HECT-type E3, and RBR-type E3.

RING-Type E3

RING-type E3s interact with charged E2 enzymes and target proteins simultaneously and transfers Ub from the E2 to the target protein directly (Yang et al., 2021 & Lorenz et al., 2013). Since E2 can directly engage the target protein, E2 is not just a carrier of Ub but can determine the specificity of Ub modification on the target substrate. It is also discovered that RING-type E3 enhances the reactivity of many E2~Ub conjugates towards aminolysis. The C-terminal of Ub is highly flexible, allowing the Ub to dynamically swing by its tail while being attached to the E2 active site. This movement results in two different states of E2~Ub: the open state and the closed state. In E3/E2~Ub structure, it is revealed that, by forming a hydrogen bond with an E2 backbone carbonyl, RING residue of E3 directs the Ub C-terminus to be in the closed state, which is the activated state for ubiquitination (Stewart et al., 2016).

HECT-Type E3

HECT-type E3s include an additional trans-thioesterification, where the activated E2 ligase transfers Ub to the active cysteine site prior to binding to the target protein (Yang et al., 2021 & Lorenz et al., 2013). HECT domains of this enzyme consist of two lobes, N-lobe carrying the E2 binding site and C-lobe containing the catalytic cysteine. During E2 binding, the two lobes adopt an open, 'L'-shaped conformation, leading to >40 Å gap between the catalytic cysteine of E2 and E3. However, after the E2 binding, the two HECT lobes adopt a closed, "T'-shaped conformation, which is enabled by a flexible hinge region connecting the two lobes, giving rise to approximately 17 Å gap between the catalytic residues of E2 and E3. As 17 Å gap is not enough for trans-thioesterification to occur, the HECT C-lobe additionally rotates and makes contacts with E2 bound ubiquitin through the conserved hydrophobic surface on the C-lobe, presumably. A remaining gap of approximately 8 Å between the catalytic cysteine of E2 and E3 can be closed by slight rotations around the flexible hinge region, finally enabling trans-thioesterification (Lorenz et al., 2013).

RBR-Type E3

RBR-type E3s contain a RING1, a central in-between-RINGs (IBR), and a RING2 domain (Yang et al., 2021). RING 1 recruits the charged E2 enzyme. Similar to HECT E3s, RBRs contain a catalytic cysteine in the RING2 domain, where the Ub is firstly transferred before ubiquitination (Yang et al., 2021 & Lorenz et al., 2013). RBR-type E3s generally undergo similar mechanisms as HECT-type E3s, but tend to ubiquitinate substrates via linear ubiquitin chain (Yang et al., 2021).

Conclusion

All eukaryotes express the enzymatic machinery to covalently modify substrate proteins with ubiquitin(s), a process called ubiquitination. Ubiquitination is catalyzed by three enzymes (E1, E2, and E3) that results in the transfer of ubiquitin, via its C-terminal glycine, onto the epsilon-amino group of a lysine residue on the substrate. Ubiquitination

affects protein stability, subcellular localization, and the ability to interact with other proteins. In recent years, studies have shown the detailed molecular mechanisms of the ubiquitination process, during which E1 and E3 undergo large conformational changes to perform distinct biochemical reactions. Although E2 is a relatively small protein, it does determine the linkage specificity of most of the ubiquitination processes.

Acknowledgments

I would like to thank my advisor for the valuable insight provided to me on this topic.

References

Damgaard R. B. volume 28, pages 423–426 (2021) *The ubiquitin system: from cell signaling to disease biology and new therapeutic opportunities* Cell Death & Differentiation

Lorenz, S., Cantor, A.J., Rape, M.& Kuriyan, J. (2013, 11:65) *Macromolecular juggling by ubiquitylation enzymes* BMC Biology

Schmelz, S and Naismith, J.H. (2009, 19:666–671) *Adenylate-forming enzymes* Current Opinion in Structural Biology

Stewart, M.D., Ritterhoff, T., Klevit, R.E., Brzovic, P.S. (2016 26:423-440) *E2 enzymes: more than just middle men* Cell Research

Yang, Q., Zhao, J., Chen, D. & Wang, Y. (2021 2:23) *E3 ubiquitin ligases: styles, structures and functions* Molecular Biomedicine

Yuan, L., Lv, Z., Adams, M.J., & Olsen, S.K. (2021, 12:2370) *Crystal structures of an E1–E2–ubiquitin thioester mimetic reveal molecular mechanisms of transthioesterification* Nature communication