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Extended Abstract
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In organic Chemistry : 2018 Chemical tools to probe protein ubiquitination- Jun Yin

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Ubiquitin (UB) is transferred through an E1-E2-E3 enzymatic cascade to the substrate proteins to regulate their stability and biological functions in the cell. The human genome encodes 2 E1s, 45 E2s, and more than 600 E3s. Together they assemble a complex network of UB transfer for the modification of cellular proteins. Currently, key questions are unsolved on how to identify ubiquitination targets of important E3s to map them on the cell signaling networks, and how UB chains of specific linkages are assembled to encode unique signals in the cell. We have developed a method that we refer to as Ã¢Â□Â□orthogonal UB transferÃ¢Â□Â□ (OUT) to untangle the complexity of protein ubiquitination networks. The key to OUT is to engineer a cascade of engineered E1, E2 and E3 enzymes (xE1, xE2, and xE3) that exclusively transfers an engineered UB (xUB) to the substrates of a xE3. We express xUB and the OUT cascade in the cell, purify xUB-conjugated proteins, and reveal their identities by proteomics. The proteins from the OUT screen are the potential substrates of the E3 in the OUT cascade. We have developed OUT cascades with HECT E3 E6AP and U-box E3s E4B and CHIP and identified new cellular circuits regulated by these E3s. To investigate the mechanism of E2-catalyzed UB chain synthesis, we have generated linkage-specific di-UB conjugates by unnatural amino acid incorporation and expressed protein ligation. The di-UB conjugates mimic the binding modes of donor and acceptor UBs at the E2 active site for UB chain synthesis. By characterizing the structure of E2-diUB conjugates, we are to reveal how E2 regulates the synthesis of UB chains of different linkages.

Ubiquitin (Ub) may be a small (8.6 kDa) regulatory protein of 76 amino acids that adopts a β-grasp fold. Ub is very conserved in eukaryotic organisms. The conjugation of ubiquitin to a target protein is named ubiquitination or ubiquitylation.1 Typically, Ub is attached to proteins through an isopeptide linkage between the C-terminal carboxylate of ubiquitin (glycine 76) and an ε-amino group of a lysine residue within the acceptor proteins. Ubiquitination is a crucial , reversible post-translational modification (PTM) in eukaryotic cells. Since its discovery within the late 1970s and early 1980s, the modification by ubiquitin has emerged as an important regulatory mechanism in most cellular processes in eukaryotes. Ubiquitination affects substrate proteins in many various ways including signaling, proteasomal degradation, altering cellular localization, modulating catalytic activity, and promoting or preventing protein interactions.2, 3 The cellular processes regulated by ubiquitination include cell cycle, transcription, trafficking, inflammation and DNA repair. Notably, many of the processes are independent of proteasome-mediated protein degradation.

Ubiquitination involves three main enzymatic steps catalyzed by ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s) (see Fig. 1).5 First, ubiquitin is activated during a two-step reaction by an E1 (ubiquitin-activating enzyme) with the consumption of ATP, forming a ubiquitin adenylate intermediate and subsequently a thioester bond between the C-terminal carboxyl of ubiquitin and therefore the site cysteine of E1.The human genome contains two E1s, i.e. UBA1 and UBA6.8 E2 catalyzes the transfer of Ub from the Ub-E1 conjugate to the site cysteine of E2 and forms the Ub-E2 conjugate through a transthioesterification reaction. The human genome possesses quite 30 different E2 enzymes. The E3 ubiquitin ligase catalyzes the ultimate step of the ubiquitination cascade by transferring Ub from the Ub-E2 conjugate to the substrate protein. E3s have substrate specificity for the E2 enzymes. The cullin-RING ligases, which constitute the most important group of E3s (around 600 members), don't form a chemical bond with Ub. Two smaller groups of E3s, the HECT ligases (around 30 members) and RBR ligases (around 12 members), form a Ub-thioester intermediate with the E3 site cystein

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The protein modifications are often either one ubiquitin protein (monoubiquitination) or a sequence of ubiquitin (polyubiquitination). Secondary ubiquitin molecules are always linked to at least one of the seven lysine residues or the N-terminal methionine of the previous ubiquitin molecule. These 'linking' residues are represented by a "K" or "M" (the one-letter aminoalkanoic acid notation of lysine and methionine, respectively) and variety , pertaining to its position within the ubiquitin molecule as in K48, K29 or M1. the primary ubiquitin molecule is covalently bound through its C-terminal carboxylate group to a specific lysine, cysteine, serine, threonine or N-terminus of the target protein. Poly-ubiquitination occurs when the C-terminus of another ubiquitin, is then linked to at least one of the seven lysine residues or the primary methionine on the previously added ubiquitin molecule, creating a sequence . This process repeats several times, resulting in the addition of several ubiquitins. Only poly-ubiquitination on defined lysines, totally on K48 and K29, is said to degradation by the proteasome (referred to because the "molecular kiss of death"), while other polyubiquitinations (e.g. on K63, K11, K6 and M1) and monoubiquitinations may regulate processes like endocytic trafficking, inflammation, translation and DNA repair

Activity-based ubiquitin probes are among the foremost crucial and versatile tools to know specificity and activity of interacting proteins and DUBs. Currently, several methods of synthesizing monoubiquitin, diubiquitin and triubiquitin ABPs are developed including solid phase synthesis and chemoenzymatic reactions. These probes have already yielded valuable information on how DUBs recognize and process polyubiquitin chains and ubiquitinated proteins. The molecular and structural diversity of polyubiquitin chains makes understanding ubiquitin signaling a challenging yet exciting undertaking. The future ubiquitin research will enjoy the arrival and continued development of ubiquitin-based probes. Ubiquitin ABPs are getting more sophisticated to incorporate polyubiquitin chains of mixed and branched linkages also as polyubiquitinated proteins. Additionally, these ABPs leave interrogation of auxiliary sites useful for development of inhibitors targeting DUBs and ubiquitin binding proteins. they're going to help to deepen our understanding of complex ubiquitin cellular signaling pathways and develop new therapies targeting the ubiquitin system.

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