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Ubiquitination Enzymes

Toshiyuki Habu and Jiyeong Kim

Abstract

Posttranslational protein modifications by mono- or polyubiquitination are involved in diverse cellular signaling pathways and tightly regulated to ensure proper function of cellular processes. Three types of enzymes, namely ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin-protein ligases (E3), contribute to ubiquitination. Combinations of E2 and E3 enzymes determine ~ the fate of their substrates via ubiquitination. The seven lysine residues of ubiquitin, Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63, can serve as attachment sites for other ubiquitin molecules. Lys48 (K48)-linked polyubiquitination facilitates recognition of the conjugated protein by proteasome molecules and subsequent proteolytic degradation of the target protein. By contrast, Lys63 (K63)-linked polyubiquitination appears to be involved in polyubiquitin signaling in critical cellular processes, such as DNA repair, regulation of the I-kappaB kinase/NF-kappaB cascade, or T cell receptor signaling, but not protein degradation. In this review, we describe the properties of ubiquitin modification enzymes and the structural interplay among these proteins.

Keywords: E1 ubiquitin, ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, E3 ubiquitin ligase

1. Introduction

Very large-scale studies of protein ubiquitination have been conducted over the past two decades. Ubiquitin modification is mediated by three types of enzyme activity, mediated out by E1 ubiquitin-activating enzymes (UBA; also referred to as UAE or E1 enzymes; EC 6.2.1.45), E2 ubiquitin-conjugating enzymes (UBC; also termed E2 ubiquitin-carrier proteins or E2 enzymes; EC 2.3.2.23), and ubiquitin-protein ligases (E3 enzymes). To better understand the molecular mechanisms underlying ubiquitin modification, this review focuses on the structural interactions between ubiquitin modification enzymes and their functions.

2. Types of ubiquitin and ubiquitin-like proteins (UBLs)

Ubiquitin is a small, highly conserved 76 amino acids polypeptide found throughout eukaryotic cells, that modifies cellular proteins. Two mammalian ubiquitin genes, *UBB* and *UBC*, encode polyubiquitin and another two genes, *RPS27A* and *UBA52* encode ubiquitins fused with ribosomal proteins [1]. Ubiquitin is produced as precursor peptides that are proteolytically processed by deubiquitinating enzymes into

active forms with C-terminal glycine residues. The C-terminal glycine (Gly76) and seven lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63) of ubiquitin are essential for ubiquitin modification. There is 96% sequence identity between human and yeast ubiquitin, and the two glycine and seven lysine residues are conserved throughout the eukaryotic kingdom.

Ubiquitin-like proteins (UBLs) do not share sequence homology with ubiquitin but also function as protein modifiers. A number of UBLs have been reported, including SUMO1/SMT3, SUMO2–4 [2–4], Neural-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8)/RUB1 [3, 5, 6], ISG15 [6, 7], ATG8/APG8 [8], ATG12/APG12 [9], URM1 [3], and homologous to ubiquitin 1 (HUB1) [10]. During protein modification by ubiquitin and UBLs, specific activating, conjugating, and ligase enzymes, catalyze attachment of the modifier to target proteins. Similar to ubiquitin, UBLs are also produced as precursors, and deubiquitinating enzymes expose their C-terminal glycine residues to activate them, although HUB1 lacks a C-terminal diglycine sequence.

The SUMO-1 protein has only 18% sequence identity with ubiquitin, but contains the $\beta\beta\alpha\beta\beta\alpha\beta$ fold structure characteristic of the ubiquitin protein family [2]. The hydrophobic cores of SUMO-1 and ubiquitin are similar; however, the overall charge surface topology of SUMO-1 differs significantly from that of ubiquitin or other UBLs [2, 11]. The selective modifications mediated by the four SUMO homologs, SUMO-1, SUMO-2, SUMO-3, and SUMO-4 [12–16], remain to be determined. In addition, SUMO has an N-terminal extension (approximately 20 amino acids) not present in ubiquitin, which is required for SUMO function [17]. A consensus motif and lysine residues involved in SUMOylation are present in SUMO-2, SUMO-3, and SUMO-4 and well-conserved; however, these SUMO proteins do not have Lys residue counterparts of ubiquitin Lys 48 and 63.

NEDD8 shows 58% sequence identity and 80% sequence similarity to ubiquitin polypeptide. By contrast, the ATG12 sequence is unrelated to that of ubiquitin, and forms a covalent protein complex with ATG5, which is required for autophagy; this reaction requires the C-terminal glycine residue of ATG12 and a lysine residue in ATG5 [18].

The ISG15/ubiquitin cross-reacting protein (*UCRP*) gene comprises two exons and encodes a 17 kDa polypeptide [19, 20]. The immature polypeptide is cleaved at its carboxy terminus, generating a mature 15 kDa product that terminates with an LRLRGG motif that is also found in ubiquitin. The tertiary structure of ISG15 also resembles ubiquitin, despite having only approximately 30% sequence homology [21, 22]. ISG15 is induced by type I interferon and serves many functions, acting as an extracellular cytokine and an intracellular protein modifier [23, 24].

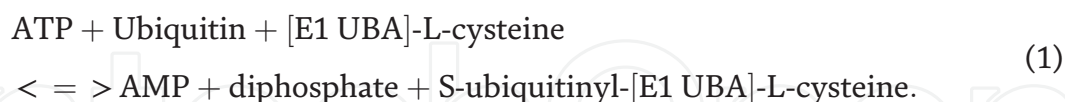
HLA-F adjacent transcript 10 (FAT10; also known as ubiquitin D) also bears two ubiquitin-like domains. HUB1 has only 22% sequence identity with ubiquitin and possesses an invariant C-terminal double-tyrosine motif, unlike the double glycine residues present in ubiquitin and other UBLs [25].

3. Ubiquitin modification enzymes

3.1 E1 ubiquitin-activating enzymes (UBA)

E1 UBA enzymes adenylate the C-terminal glycine residue (Gly 76) of ubiquitin polypeptides, coupling them with ATP. The C-terminal ubiquitin polypeptide glycine

residue is linked to AMP via an acyl-phosphate bond, and the adenylated ubiquitin polypeptide is linked with the sulfhydryl side chain of a cysteine residue (Cys 632 in human UBA1) in the E1 enzyme catalytic center. A thioester intermediate (S-ubiquitinyl-(E1 UAE)-L-Cys) is synthesized in this two-step reaction, along with AMP and diphosphate (Reaction (1)).



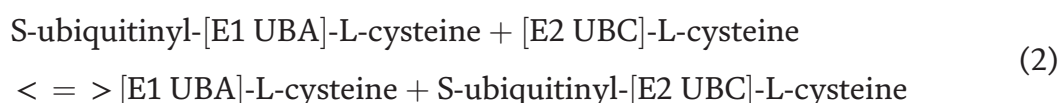
In human, ten UBA orthologues have been identified that can activate ubiquitin or UBLs. Ubiquitin-like modifier-activating enzyme 1 (UBA1) is mainly responsible for ubiquitin-activation and can also activate the NEDD8 UBL peptide [26–28]. UBA2 (or UBLE1B) is also known as SUMO-activating enzyme subunit 2 (SAE2), and activates the SUMO UBL peptide as heterodimer with SAE1 [29]. UBA3 (or UBE1C) encodes the NEDD8-activating enzyme E1 catalytic subunit and forms a heterodimer with NAE1 (or APPBP1, an amyloid-beta precursor protein binding protein), which activates NEDD8. UBA5 activates UFM1 (ubiquitin-fold modifier 1) [30], while UBA6 (alternatively UBE1L2) is an E1 enzyme involved in UBL activation [31, 32]. Autophagy related 7 (ATG7) is an E1 enzyme for UBLs including ATG12 and ATG8. NEDD8-activating enzyme E1 regulatory subunit (NAE1 or APPBP1) is an E1 enzyme for NEDD8, along with UBA3 [33]. Ubiquitin-like modifier-activating enzyme 1 Y (UBA1Y) is encoded by the Y chromosome and expressed specifically during spermatogenesis [34–37]. The UBL, FAT10, is activated by UBA1 and UBA6. UBA7 is induced by interferon- α and β and involved in ISG15 induction.

UBA structures consist of an adenylation domain that interacts with ATP and UBLs, a catalytic domain comprising a Cys residue that binds to UBLs, and a C-terminal ubiquitin fold domain (UFD) required for binding to E2 enzymes.

In a study of mammalian UBA1 with a temperature-sensitive (ts) mutation, cells expressing the ts-UBA1 mutant exhibited cell cycle arrest at the G₂/M phase transition, as well as dramatically decreased ubiquitin conjugation [38, 39]. UBA1-knockdown in human cells also leads to reduced cell proliferation [40]. Furthermore, cells expressing the ts-UBA1 mutant show reduced receptor tyrosine kinase endocytosis and degradation [41]. In addition, mice lacking UBA3 are characterized by a mitotic defect in G₁/G₀ transition, that causes accumulation of SCF ligase targets, including Cyclin E and β -catenin.

3.2 E2 ubiquitin-conjugating enzymes (UBC)

E2 UBC enzymes transthiolate activated ubiquitin from S-ubiquitinyl-[E1 UBA] to themselves. A thioester linkage is formed between an E2 UBC and ubiquitin via the C-terminal glycine of ubiquitin, and the sulfhydryl side chain of a Cys residue in the E2 UBC catalytic center [42] (Reaction (2)).



The transthiolation reaction involving S-ubiquitinyl-[E1 UBA]-L-cysteine and E2 UBC is strongly stimulated by occupancy of the nucleotide-binding site by either adenylated ubiquitin or ATP alone [43]. Ubiquitin transfer to the target protein is

assisted by E3 ubiquitin ligases. Homologous to E6-AP C-terminus (HECT) domain family E3 ligases transfer ubiquitin to the target via a Cys residue in the E3 ligase. By contrast, Really Interesting New Gene (RING) family E3 ligases transfer ubiquitin directly to target proteins. The properties of specific E2 UBC enzymes determine the ubiquitin moiety and substrate specificity of E3 ligases. Indeed, the specificity of interactions with E2 reflect E3 substrate specificity. Amino acids surrounding the Cys residue are evolutionarily conserved among E2 UBCs, and referred to as the ubiquitin-conjugating (UBC) domain or the core catalytic domain [44]. The UBC domain folds an N-terminal helix (H1), a four-stranded β -meander structure (S1–S4), a short 3_{10} -helix (H2), and three C-terminal helices (H3–H5) [45, 46]. Amino acid sequence variations in the UBC domain contribute to specific interactions with E1 UBAs, E3 ligases, and target proteins [47–51].

E2 UBCs are divided into four classes based on structural differences [52]: class I E2 enzymes consist of only a UBC domain; class II E2 enzymes contain additional C-terminal extension residues; class III E2 enzymes have N-terminal extension residues; and class IV E2s have both N- and C-terminal extensions. Class II UBC2 and UBC3 proteins have acidic C-terminal extensions, which mediate a preference for binding to basic substrates, including histones [53–55]. The acidic extension is also required to contact basic canyon residues of the Cul1 subunit of the SCF RING subcomplex (ROC1-CUL1) [56–58]. UBC6 processes C-terminal extensions, to promote ER localization [59, 60]. Class II UBCs include: E2-25K (yeast UBC1) [61], UBC4 [62], UBCH6, UBCH7 [63], UBE2E1 [64], UBE2E2 [65], and UBE2E3 [66, 67].

UBL-specific E2 UBC enzymes process proteins for ubiquitin-like modification. UBC9 is an E2 UBC enzyme specific for the UBL, SUMO, and binds directly to SUMO substrates through a specific short consensus amino acid motif, Y-K-X-[D/E], where Y is any bulky hydrophobic amino acid, including isoleucine, leucine, or valine; K is the lysine residue which is modified by SUMO; X is any residue; D is aspartic acid; and E is glutamic acid [68]. UBC9 contains N- and C-terminal extension residues within the UBC domain, and non-conserved residues in the H1 helix and the insertion β -sheet (S1/2) are required for both interaction with UBA enzymes and formation of the SUMO-thioester bond [69, 70]. ATG3/AUT1 is a dedicated E2 UBC for ATG8 [71]. UBC12 is an E2 UBC specific for NEDD8, which interacts with the NEDD8 E1 UBA via its UBC domain [72, 73], and includes a unique N-terminal region that docks to the E1 enzyme, UBA3, but not to other UBAs. The E2 for ISG15, UBCH8, takes part in reactions involving both UBEL1, the E1 for ISG15, and UBA1, the E1 for ubiquitin [7].

3.3 E3 ubiquitin ligases

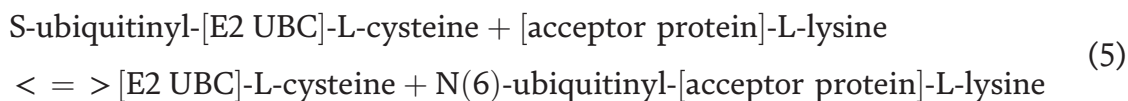
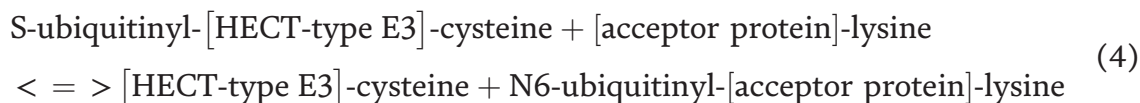
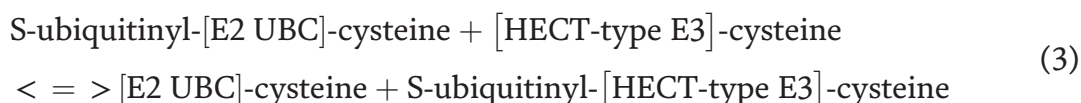
E3 ubiquitin ligases are also referred to as ubiquitin-protein ligases, E3 ligases, or E3 enzymes. Ubiquitin is covalently bonded to the ϵ -amino group of a lysine residue within the substrate protein via an isopeptide bond. The last step in this binding is mediated by E3 ubiquitin ligases, which determine the substrate specificity by \sim to target proteins. E3 ubiquitin ligases transfer ubiquitin linked with a UBC to the ϵ -amino group of a lysine residue of the target protein. An isopeptide bond is formed between the C-terminal glycine residue of ubiquitin and an ϵ -amino group of a lysine residue in the target protein.

E3 ubiquitin ligases are divided into three major classes: HECT type (Section 3.3.1), RING-type (Section 3.3.3), and U-box E3 ligases (Section 3.3.4). HECT type E3 ubiquitin ligases form a thioester intermediate with the active-site cysteine of the E3, following the formation of an isopeptide bond between the C-terminal glycine residue

of ubiquitin and the ϵ -amino group of a lysine residue in the target protein. RBR-type E3 ubiquitin ligases (Section 3.3.2) mediate similar reactions to HECT type E3 ligases. RING-type and U-box E3 ligases mediate different reactions from HECT and RBR-type E3 ligases, in which ubiquitin is transferred from ubiquitinyl-UBC directly to the target protein without formation of a thioester intermediate. Multi-subunit RING-type E3 ligases (Section 3.3.3.1) form complexes with a scaffold protein and contain recognition modules that bind to substrates.

3.3.1 HECT-type E3 ubiquitin transferases (EC 2.3.2.26)

HECT-type E3 ligases transfer ubiquitin from an E2 ubiquitin-conjugating enzyme (EC 2.3.2.23) to a cysteine residue in the HECT domain in the C-terminal region of an E3 ligase (Reaction (3)). The activated ubiquitin from S-ubiquitinyl-[E3 ligase]-L-cysteine is transferred from the intermediate to the target protein (Reaction (4)). The C-terminal glycine residue of the received ubiquitin is linked with the ϵ -amino chain of a lysine residue of the acceptor protein, forming an isopeptide bond. Importantly, the HECT domain forms a thioester intermediate with ubiquitin, unlike other E3 ligases.



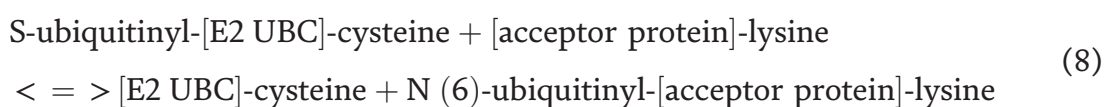
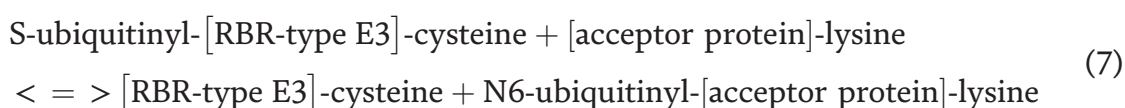
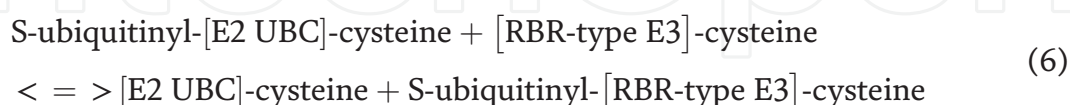
HECT type E3 ligases catalyze a thioester bond between a C-terminal glycine residue of ubiquitin and themselves and then transfer the ubiquitin to a substrate protein. HECT type E3 ligase family proteins possess a well-conserved, approximately 350 residue, catalytic HECT domain close to their C-terminal region [74, 75]. The HECT domain has a bi-lobal structure comprising an approximately 250 residue N-lobe, required for the binding to UBC-ubiquitin complex, and a C-lobe of around 100 residues, required for ubiquitin transfer [74, 76, 77]. Various linker sequences between the two HECT domain lobes mediate the properties of HECT type E3 ligases in accepting ubiquitin from E2 enzymes and transferring it to a target substrate.

The HECT type E3 ligase, E6-AP, can ubiquitinate p53 in the presence of human papillomavirus E6 protein [75, 78, 79], and another HECT E3 enzyme NEDD4 ubiquitinates SMAD proteins, thereby regulating transcription factors mediating TGF β signaling [80], the P63 tumor antigen [81], and MDM2 [82]. HECW1 [83], HECW2 [84], WWP1 [85], HERC1 [86], HERC2 [87], and ITCH [88] also belong to the HECT type E3 ubiquitin ligase family.

3.3.2 RBR-type E3 ubiquitin transferase (EC 2.3.2.31)

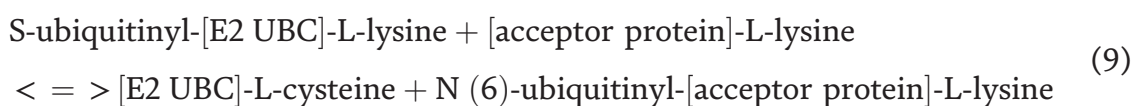
RBR-type E3 ubiquitin transferases possess two RING finger domains, each of which is separated by an internal IBR (In Between RING) motif. These E3 ligases bind to the Cullin-RING ubiquitin Ligase (CRL) complex (see Cullin-type E3 NDD8

transferase), within which a neddylated cullin scaffold protein and a substrate recognition module are required for ubiquitin transfer. The first RING domain binds S-ubiquitinyl- [E2 UBC]-cysteine and transfers the ubiquitin to an internal Cys residue in the second RING domain (Reaction (6)), followed by transfer of the ubiquitin from the Cys residue in the second RING domain to a Lys in the acceptor protein (Reaction (7)). RBR-type ligases stimulate a cycling ubiquitination reaction via the S-ubiquitinyl-[E2 UBC]-cysteine in the first RING domain [88]. RBR-type ligase activity depends on the neddylation of the cullin protein in the CRL complex. RBR-type E3 ubiquitin ligases include Parkin, Parc, RNF19, RNF144, RNF216 RFA1 HOIP, and HHARI [89, 90].



3.3.3 RING-type E3 ubiquitin transferases (EC 2.3.2.27)

RING E3 ubiquitin ligases (also referred to as RING E3 ligases or ubiquitin transferase RING E3 enzymes) transfer ubiquitin peptides directly from a ubiquitinyl-E2 UBC enzyme to an acceptor protein. The ϵ -amino group of a lysine residue of the target protein forms an isopeptide bond with the C-terminal glycine residue of ubiquitin (Reaction (9)). Unlike HECT E3 ligases, the RING-E3 domain does not create a catalytic thioester intermediate with ubiquitin through a Cys residue.



Human proteome analysis has identified approximately 580 genes encoding putative RING-type ubiquitin E3 ligase family proteins, which is more than the number of protein kinase genes (518) [91]. Among RING-type E3 ligase genes, 309 and 270 encode single and multi-subunit RING-type E3 ligase molecules, respectively. While RING-type E3 ubiquitin ligase family proteins do not form thioester intermediates with ubiquitin, they function as a scaffold for ubiquitin-charged UBC and the substrate. RING-type E3 ligases contain both a RING domain and a substrate-binding site, and almost half the RING proteins belong to multisubunit RING-type E3 ligases, which require an additional subunit for substrate recognition (see multisubunit RING-type ubiquitin ligases).

The RING domain was initially thought to function as a DNA binding domain because of the discovery of RING domain-containing proteins with DNA binding activity [92, 93]. RING-type E3 ligases were subsequently identified as interacting partners of the human E2 ubiquitin-conjugating enzyme UBCH5 [94], which has self-ubiquitination activity that depends on its RING domain sequence. The canonical RING domain structure consists of a Zn^{2+} -coordination complex and a series of Cys and His residues and mediates E2-dependent ubiquitylation. The coordination

complex with two zinc ions forms a cross-brace structure. RING finger domains have consensus sequences that are classified into two different types, C₃HC₄-type (RING-HC) and C₃H₂C₃-type (RING-H2), according to the cysteine/histidine arrangement (where C = Cys and H = His) [95]. The C₃HC₅-type RING domain has different properties from the C₃HC₄ RING-HC finger [96], Casitas B-lineage Lymphoma (c-Cbl), which is a RING-HC type ligase. Ubiquitination activity modulates receptor tyrosine kinase signaling [97] and structural analysis of the c-Cbl-UbcH7-substrate tertiary complex showed that the interaction surface of the UbcH7 E2 enzyme is commonly used by both c-Cbl and HECT-type E3 ligases, where c-Cbl binds UbcH7 using both its RING domain and linker helix structure [50]. The amino acid residues involved in the interaction are structurally similar between E2 enzymes and E3 ligases.

BRCA1 forms a heterodimer with the RING-type ligase BARD. The dimerization of two RING-type E3 ligases results in upregulation of ubiquitination activity. By biochemical approaches, UbcH5c and UbcH7 enzymes were identified as candidate E2 enzymes for the BRCA1-BARD complex. Christensen et al. developed an excellent method for identifying E2-E3 pairing [98, 99], using a BRCA1-BARD fusion protein; BRCA1 can synthesize specific polyubiquitin chain linkages, depending on the presence of a paired E2 enzyme [98]. This approach has increased the identification of E2-E3 pairs; for example, RNF213, a RING-HC type E3 ligase and its paired UBC13E2 enzyme were identified using this method [100]. UBCH5b mutants, which can bind to E3 ligase, exhibit defective stimulation by E3 ligases [101]. Ubiquitin-charged E2 is conformationally activated by binding to the RING domain [101–103]. Furthermore, the interaction between E1 and E2 enzymes can direct substrate specificity, ubiquitin transfer, and polyubiquitin chain linkages.

Some RING-type ubiquitin ligase family members form hetero- or homo-multimers through the RING domain or its surrounding region. RING-RING complexes, including MDM2-HMDX, BRCA1-BARD1, and RING1-BMI1, form heterodimers. In heterodimers, one partner (HDMX, BRAD1, and BMI1) lacks ubiquitin ligase activity, while the other partner (MDM2, BRCA1, and RING1) has E2 UBC binding activity. Heterodimer formation leads to stabilization of E2-E3 binding, and in dimerizing E3 ligases, the five C-terminal residues of the RING domain are essential for both dimer formation and E3 activity [104–106].

TRAF2, cellular inhibitor of apoptosis (cIAP; officially known as BIRC2), SIAH, BIRC7, and RNF213 form homodimers [100, 107–111]. Dimeric BIRC7 recruits UBCH5B-ubiquitin and optimizes the donor ubiquitin configuration for transfer [112]. Homo- and hetero-dimerization of RING-type ubiquitin ligases may stabilize their interactions with ubiquitin-charged UBC E2 enzymes and optimize ubiquitin transfer activity.

3.3.3.1 Multisubunit RING protein complexes

Enzymes of the RING-type E3 ubiquitin ligase family do not bind directly to a substrate, but rather form a complex with a cullin scaffold protein and substrate recognition modules, referred to as CRL complexes. The SCF complex (SKP, Cullin, F-box containing complex) and anaphase-promoting complex/cyclosome (APC/C) (anaphase-promoting complex/cyclosome) are two major multisubunit RING containing complexes.

Ubiquitination by SCF and APC/C are implicated in the degradation of cell cycle proteins [113–116]. APC/C regulates mitosis and entry into the G1 phase of the cell cycle, and SCF controls S phase progression.

SCF E3 complexes comprise at least four different subunits, including the F-box protein, SKP adaptor protein, Cullin scaffold protein, and Rbx RING-type E3 ligase [116–119]. The F-box motif is a protein–protein interaction motif comprising approximately 50 amino acid residues. There is low sequence identity among F-box proteins, which recognize and bind substrate and bridge connections between adaptor proteins (including SKP1) and substrates. Phosphorylation of F-box proteins regulates their interactions with substrates. The SKP adaptor proteins, SKP2 (S-phase kinase-associated protein 2), β -TrCP (beta-transducin repeat-containing protein), FBW7, and FBXO4 are F-box proteins involved in cell-cycle regulation. Cullins are scaffold proteins for ubiquitin ligases; CUL1 is a subunit of the SCF complex, and the Cullin-homology domain at its C-terminus interacts with RING E3 ligases while the N-terminal region can interact with the adaptor protein, SKP2. Cullin family members function as adaptors for multisubunit RING-type E3 ligase complexes. The adaptor proteins SKP1 and CUL1 and the RING-type E3 ligase RBX1 form the CRL catalytic core complex.

APC/C is a multisubunit RING-type E3 ligase containing approximately 13 subunits. The Cullin subunit protein, APC2, and the RING H2 type E3 ligase, APC11, form the catalytic core domain [120–122]. TPR (tetratricopeptide residue) motif-containing subunits, including CDC16, CDC27, CDC23, and APC5, are thought to function as scaffold assembling proteins. Two co-activators, CDC20 (cell division cycle homologue 20) and FZR/CDH1 (Fzy-related/cell division cycle 20 related 1), bind to the CDC27 subunit of APC/C through their WD40 repeat and determine APC/C substrate specificity dependent on cell cycle to stages [115, 118, 123–128]. The APC10 subunit contributes to optimal co-activator-dependent substrate recognition and substrate affinity [129–131]. APC/C-mediated ubiquitination depends on destruction box, KEN box, and CRY box sequences in the substrate [132–140]. Assembly of these co-activators into the APC/C complex in G₁ or M phase during cell cycle is regulated by phosphorylation [141–144].

3.3.4 U-Box E3 ubiquitin ligases

The U-box domain displays a similar three-dimensional structure to the RING domain [145]. The U-box domain shows similarity to UFD2, which has a multiubiquitin chain elongation activity (known as E4 activity) [146]. Unlike the RING domain, the U-box domain does not form a coordination complex consisting of a central zinc ion through Cys residues; rather, the U-box domain structure is maintained by hydrogen bonding. The U-box domain has ubiquitin ligase activity, and the U-box protein, carboxyl terminus of HSC70-interacting protein (CHIP), also has E4 activity and includes tetratricopeptide repeat and U-box domains. The C-terminal U-box domain interacts with the molecular chaperones HSC70, HSP70, and HSP90 [147], in the presence of unfolded or misfolded proteins, where CHIP regulates protein quality control [148, 149].

U-box proteins have various structures; for example, ARC1, CMPG1, PUB13, and PUB20 contain armadillo which represents approximately 40 amino acids tandem repeats sequence. PUB23 has a serine/threonine kinase domain while PUB59 and PUB60 have WD40 repeats. These domains may coordinate the function of ubiquitin ligase activity by the U-box domain.

4. Conclusions

Research conducted over several decades has uncovered the cellular and biochemical functions involved in ubiquitin modification. Protein–protein networks and

studies of complex structures have contributed to unraveling the biochemical mechanisms underlying ubiquitin modification. Identification of physiological E2 UBC-E3 ligase pairings has facilitated understanding of modification-types and associations. Deep understanding of the structures and biochemical processes involved in ubiquitin modification has contributed to determination of E3 ligase-substrate pairing and network construction. RING-type ubiquitin ligases comprise the largest gene family and are associated with various cellular processes and several diseases. Fundamental questions remain to be answered regarding the biological functions served by ubiquitin modification. Extensive further study of enzymes involved in ubiquitination and related processes has potential to contribute to the understanding of the pathogenesis of several diseases.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations


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