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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 a 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

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Notes/thanks/other declarations

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References

- [1] Redman KL, Rechsteiner M. 1989 Identification of the long ubiquitin extension as ribosomal protein S27a. *Nature* 338, 438–440. (doi:10.1038/338438a0)
- [2] Bayer P, Arndt A, Metzger S, Mahajan R, Melchior F, Jaenicke R, Becker J. 1998 Structure determination of the small ubiquitin-related modifier SUMO-1. *J Mol Biol* 280, 275–286. (doi:10.1006/jmbi.1998.1839)
- [3] Jentsch S, Pyrowolakis G. 2000 Ubiquitin and its kin: how close are the family ties? *Trends Cell Biol* 10, 335–342. (doi:10.1016/s0962-8924(00)01785-2)
- [4] Schwartz DC, Hochstrasser M. 2003 A superfamily of protein tags: ubiquitin, SUMO and related modifiers. *Trends Biochem Sci* 28, 321–328. (doi:10.1016/s0968-0004(03)00113-0)
- [5] Larsen CN, Wang H. 2002 The Ubiquitin Superfamily: Members, Features, and Phylogenies. *J Proteome Res* 1, 411–419. (doi:10.1021/pr025522n)
- [6] Kerscher O, Felberbaum R, Hochstrasser M. 2006 Modification of Proteins by Ubiquitin and Ubiquitin-Like Proteins. *Annu Rev Cell Dev Bi* 22, 159–180. (doi:10.1146/annurev.cellbio.22.010605.093503)
- [7] Arimoto K-I, Konishi H, Shimotohno K. 2008 UbcH8 regulates ubiquitin and ISG15 conjugation to RIG-I. *Mol Immunol* 45, 1078–1084. (doi:10.1016/j.molimm.2007.07.021)
- [8] Tsukada M, Ohsumi Y. 1993 Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *Febs Lett* 333, 169–174. (doi:10.1016/0014-5793(93)80398-e)
- [9] Schulman BA, Harper JW. 2009 Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nat Rev Mol Cell Bio* 10, 319–331. (doi:10.1038/nrm2673)
- [10] Dittmar GAG, Wilkinson CRM, Jedrzejewski PT, Finley D. 2002 Role of a Ubiquitin-Like Modification in Polarized Morphogenesis. *Science* 295, 2442–2446. (doi:10.1126/science.1069989)
- [11] Gill G. 2004 SUMO and ubiquitin in the nucleus: different functions, similar mechanisms? *Gene Dev* 18, 2046–2059. (doi:10.1101/gad.1214604)
- [12] Azuma Y, Arnaoutov A, Dasso M. 2003 SUMO-2/3 regulates topoisomerase II in mitosis. *J Cell Biology* 163, 477–487. (doi:10.1083/jcb.200304088)
- [13] Saitoh H, Hinchey J. 2000 Functional Heterogeneity of Small Ubiquitin-related Protein Modifiers SUMO-1 versus SUMO-2/3. *J Biol Chem* 275, 6252–6258. (doi:10.1074/jbc.275.9.6252)
- [14] Melchior F. 2000 SUMO-NONCLASSICAL UBIQUITIN. *Annu Rev Cell Dev Bi* 16, 591–626. (doi:10.1146/annurev.cellbio.16.1.591)
- [15] Vertegaal ACO, Ogg SC, Jaffray E, Rodriguez MS, Hay RT, Andersen JS, Mann M, Lamond AI. 2004 A Proteomic Study of SUMO-2 Target Proteins. *J Biol Chem* 279, 33791–33798. (doi:10.1074/jbc.m404201200)
- [16] Zhao Y, Kwon SW, Anselmo A, Kaur K, White MA. 2004 Broad Spectrum Identification of Cellular Small Ubiquitin-related Modifier (SUMO) Substrate Proteins. *J Biol Chem* 279, 20999–21002. (doi:10.1074/jbc.m401541200)

- [17] Bylebyl GR, Belichenko I, Johnson ES. 2003 The SUMO Isopeptidase Ulp2 Prevents Accumulation of SUMO Chains in Yeast. *J Biol Chem* 278, 44113–44120. (doi: 10.1074/jbc.m308357200)
- [18] Klionsky DJ, Ohsumi Y. 1999 VACUOLAR IMPORT OF PROTEINS AND ORGANELLES FROM THE CYTOPLASM. *Annu Rev Cell Dev Bi* 15, 1–32. (doi:10.1146/annurev.cellbio.15.1.1)
- [19] Blomstrom DC, Fahey D, Kutny R, Korant BD, Knight E. 1986 Molecular characterization of the interferon-induced 15-kDa protein. Molecular cloning and nucleotide and amino acid sequence. *J Biological Chem* 261, 8811–8816.
- [20] Dzimianski JV, Scholte FEM, Bergeron É, Pegan SD. 2019 ISG15: It's Complicated. *J Mol Biol* 431, 4203–4216. (doi:10.1016/j.jmb.2019.03.013)
- [21] Recht M, Borden EC, Knight E. 1991 A human 15-kDa IFN-induced protein induces the secretion of IFN-gamma. *J Immunol Baltim Md* 1950 147, 2617–2623.
- [22] Swaim CD, Scott AF, Canadeo LA, Huibregtse JM. 2017 Extracellular ISG15 Signals Cytokine Secretion through the LFA-1 Integrin Receptor. *Mol Cell* 68, 581–590.e5. (doi:10.1016/j.molcel.2017.10.003)
- [23] D'Cunha J, Knight E, Haas AL, Truitt RL, Borden EC. 1996 Immunoregulatory properties of ISG15, an interferon-induced cytokine. *Proc National Acad Sci* 93, 211–215. (doi: 10.1073/pnas.93.1.211)
- [24] Knight E, Cordova B. 1991 IFN-induced 15-kDa protein is released from human lymphocytes and monocytes. *J Immunol Baltim Md* 1950 146, 2280–2284.
- [25] Loeb KR, Haas AL. 1994 Conjugates of ubiquitin cross-reactive protein distribute in a cytoskeletal pattern. *Mol Cell Biol* 14, 8408–8419. (doi:10.1128/mcb.14.12.8408)
- [26] Moudry P, Lukas C, Macurek L, Hanzlikova H, Hodny Z, Lukas J, Bartek J. 2014 Ubiquitin-activating enzyme UBA1 is required for cellular response to DNA damage. *Cell Cycle* 11, 1573–1582. (doi:10.4161/cc.19978)
- [27] Correale S *et al.* 2014 Structural Model of the hUbA1-UbcH10 Quaternary Complex: In Silico and Experimental Analysis of the Protein-Protein Interactions between E1, E2 and Ubiquitin. *Plos One* 9, e112082. (doi: 10.1371/journal.pone.0112082)
- [28] Leidecker O, Matic I, Mahata B, Pion E, Xirodimas DP. 2012 The ubiquitin E1 enzyme Ube1 mediates NEDD8 activation under diverse stress conditions. *Cell Cycle* 11, 1142–1150. (doi:10.4161/cc.11.6.19559)
- [29] Okuma T, Honda R, Ichikawa G, Tsumagari N, Yasuda H. 1999 In Vitro SUMO-1 Modification Requires Two Enzymatic Steps, E1 and E2. *Biochem Bioph Res Co* 254, 693–698. (doi:10.1006/bbrc.1998.9995)
- [30] Komatsu M *et al.* 2004 A novel protein-conjugating system for Ufm1, a ubiquitin-fold modifier. *Embo J* 23, 1977–1986. (doi:10.1038/sj.emboj.7600205)
- [31] Pelzer C, Kassner I, Matentzoglou K, Singh RK, Wollscheid H-P, Scheffner M, Schmidtke G, Groettrup M. 2007 UBE1L2, a Novel E1 Enzyme Specific for Ubiquitin. *J Biol Chem* 282, 23010–23014. (doi:10.1074/jbc.c700111200)
- [32] Jin J, Li X, Gygi SP, Harper JW. 2007 Dual E1 activation systems for ubiquitin

differentially regulate E2 enzyme charging. *Nature* 447, 1135–1138. (doi:10.1038/nature05902)

[33] Bohnsack RN, Haas AL. 2003 Conservation in the Mechanism of Nedd8 Activation by the Human AppBp1-Uba3 Heterodimer. *J Biol Chem* 278, 26823–26830. (doi:10.1074/jbc.m303177200)

[34] Ayusawa D, Kaneda S, Itoh Y, Yasuda H, Murakami Y, Sugasawa K, Hanaoka F, Seno T. 1992 Complementation by a Cloned Human Ubiquitin-Activating Enzyme E1 of the S-Phase-Arrested Mouse FM3A Cell Mutant with Thermolabile E1. *Cell Struct Funct* 17, 113–122. (doi:10.1247/csf.17.113)

[35] Cook JC, Chock PB. 1992 Isoforms of mammalian ubiquitin-activating enzyme. *J Biological Chem* 267, 24315–24321.

[36] Handley PM, Mueckler M, Siegel NR, Ciechanover A, Schwartz AL. 1991 Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1. *Proc National Acad Sci* 88, 258–262. (doi:10.1073/pnas.88.1.258)

[37] Lévy N, Navarro A, Bishop CE, Mitchell MJ. 2000 The ubiquitin-activating enzyme E1 homologous genes on the mouse Y Chromosome (Ube1y) represent one functional gene and six partial pseudogenes. *Mamm Genome* 11, 164–168. (doi:10.1007/s003350010031)

[38] Finley D, Ciechanover A, Varshavsky A. 1984 Thermolability of ubiquitin-activating enzyme from the mammalian cell cycle mutant ts85. *Cell* 37, 43–55. (doi:10.1016/0092-8674(84)90299-x)

[39] Ciechanover A, Finley D, Varshavsky A. 1985 Mammalian cell

cycle mutant defective in intracellular protein degradation and ubiquitin-protein conjugation. *Prog Clin Biol Res* 180, 17–31.

[40] Schlabach MR *et al.* 2008 Cancer Proliferation Gene Discovery Through Functional Genomics. *Science* 319, 620–624. (doi:10.1126/science.1149200)

[41] Strous GJ, Kerkhof P, Govers R, Ciechanover A, Schwartz AL. 1996 The ubiquitin conjugation system is required for ligand-induced endocytosis and degradation of the growth hormone receptor. *Embo J* 15, 3806–3812. (doi:10.1002/j.1460-2075.1996.tb00754.x)

[42] David Y, Ziv T, Admon A, Navon A. 2010 The E2 Ubiquitin-conjugating Enzymes Direct Polyubiquitination to Preferred Lysines. *J Biol Chem* 285, 8595–8604. (doi:10.1074/jbc.m109.089003)

[43] Pickart CM, Kasperek EM, Beal R, Kim A. 1994 Substrate properties of site-specific mutant ubiquitin protein (G76A) reveal unexpected mechanistic features of ubiquitin-activating enzyme (E1). *J Biological Chem* 269, 7115–7123.

[44] Burroughs AM, Jaffee M, Iyer LM, Aravind L. 2008 Anatomy of the E2 ligase fold: Implications for enzymology and evolution of ubiquitin/Ub-like protein conjugation. *J Struct Biol* 162, 205–218. (doi:10.1016/j.jsb.2007.12.006)

[45] Cook WJ, Jeffrey LC, Carson M, Chen Z, Pickart CM. 1992 Structure of a diubiquitin conjugate and a model for interaction with ubiquitin conjugating enzyme (E2). *J Biol Chem* 267, 16467–16471. (doi:10.1016/s0021-9258(18)42026-1)

[46] Cook WJ, Jeffrey LC, Xu Y, Chau V. 1993 Tertiary structures of class I ubiquitin-conjugating enzymes are

highly conserved: Crystal structure of yeast Ubc4. *Biochemistry-us* 32, 13809–13817. (doi:10.1021/bi00213a009)

[47] Hamilton KS, Ellison MJ, Barber KR, Williams RS, Huzil JT, McKenna S, Ptak C, Glover M, Shaw GS. 2001 Structure of a Conjugating Enzyme-Ubiquitin Thiolester Intermediate Reveals a Novel Role for the Ubiquitin Tail. *Structure* 9, 897–904. (doi:10.1016/s0969-2126(01)00657-8)

[48] Dominguez C, Bonvin AMJJ, Winkler GS, Schaik FMA van, Timmers HThM, Boelens R. 2004 Structural Model of the UbcH5B/CNOT4 Complex Revealed by Combining NMR, Mutagenesis, and Docking Approaches. *Structure* 12, 633–644. (doi:10.1016/j.str.2004.03.004)

[49] Huang L, Kinnucan E, Wang G, Beaudenon S, Howley PM, Huibregtse JM, Pavletich NP. 1999 Structure of an E6AP-UbcH7 Complex: Insights into Ubiquitination by the E2-E3 Enzyme Cascade. *Science* 286, 1321–1326. (doi:10.1126/science.286.5443.1321)

[50] Zheng N, Wang P, Jeffrey PD, Pavletich NP. 2000 Structure of a c-Cbl-UbcH7 Complex RING Domain Function in Ubiquitin-Protein Ligases. *Cell* 102, 533–539. (doi:10.1016/s0092-8674(00)00057-x)

[51] Bernier-Villamor V, Sampson DA, Matunis MJ, Lima CD. 2002 Structural Basis for E2-Mediated SUMO Conjugation Revealed by a Complex between Ubiquitin-Conjugating Enzyme Ubc9 and RanGAP1. *Cell* 108, 345–356. (doi:10.1016/s0092-8674(02)00630-x)

[52] Jentsch S. 1992 The Ubiquitin-Conjugation System. *Annu Rev Genet* 26, 179–207. (doi:10.1146/annurev.ge.26.120192.001143)

[53] Sharon G, Raboy B, Parag HA, Dimitrovsky D, Kulka RG. 1991 RAD6 gene product of *Saccharomyces cerevisiae* requires a putative ubiquitin protein ligase (E3) for the ubiquitination of certain proteins. *J Biological Chem* 266, 15890–15894.

[54] RABOY B, KULKA RG. 1994 Role of the C-terminus of *Saccharomyces cerevisiae* ubiquitin-conjugating enzyme (Rad6) in substrate and ubiquitin-protein-ligase (E3-R) interactions. *Eur J Biochem* 221, 247–251. (doi:10.1111/j.1432-1033.1994.tb18735.x)

[55] Kolman CJ, Toth J, Gonda DK. 1992 Identification of a portable determinant of cell cycle function within the carboxyl-terminal domain of the yeast CDC34 (UBC3) ubiquitin conjugating (E2) enzyme. *Embo J* 11, 3081–3090. (doi:10.1002/j.1460-2075.1992.tb05380.x)

[56] Kleiger G, Saha A, Lewis S, Kuhlman B, Deshaies RJ. 2009 Rapid E2-E3 Assembly and Disassembly Enable Processive Ubiquitylation of Cullin-RING Ubiquitin Ligase Substrates. *Cell* 139, 957–968. (doi:10.1016/j.cell.2009.10.030)

[57] Kleiger G, Hao B, Mohl DA, Deshaies RJ. 2009 The Acidic Tail of the Cdc34 Ubiquitin-conjugating Enzyme Functions in Both Binding to and Catalysis with Ubiquitin Ligase SCFCdc4. *J Biol Chem* 284, 36012–36023. (doi:10.1074/jbc.m109.058529)

[58] Choi Y-S, Wu K, Jeong K, Lee D, Jeon YH, Choi B-S, Pan Z-Q, Ryu K-S, Cheong C. 2010 The Human Cdc34 Carboxyl Terminus Contains a Non-covalent Ubiquitin Binding Activity That Contributes to SCF-dependent Ubiquitination. *J Biol Chem* 285, 17754–17762. (doi:10.1074/jbc.m109.090621)

[59] Sommer T, Jentsch S. 1993 A protein translocation defect linked to ubiquitin

conjugation at the endoplasmic reticulum. *Nature* 365, 176–179. (doi:10.1038/365176a0)

[60] Yuan L, Lv Z, Adams MJ, Olsen SK. 2021 Crystal structures of an E1–E2–ubiquitin thioester mimetic reveal molecular mechanisms of transthioesterification. *Nat Commun* 12, 2370. (doi:10.1038/s41467-021-22598-y)

[61] Merkle N, Shaw GS. 2004 Solution Structure of the Flexible Class II Ubiquitin-conjugating Enzyme Ubc1 Provides Insights for Polyubiquitin Chain Assembly. *J Biol Chem* 279, 47139–47147. (doi:10.1074/jbc.m409576200)

[62] Matuschewski K, Hauser H-P, Treier M, Jentsch S. 1996 Identification of a Novel Family of Ubiquitin-conjugating Enzymes with Distinct Amino-terminal Extensions. *J Biol Chem* 271, 2789–2794. (doi:10.1074/jbc.271.5.2789)

[63] Nuber U, Schwarz S, Kaiser P, Schneider R, Scheffner M. 1996 Cloning of Human Ubiquitin-conjugating Enzymes UbcH6 and UbcH7 (E2-F1) and Characterization of Their Interaction with E6-AP and RSP5. *J Biol Chem* 271, 2795–2800. (doi:10.1074/jbc.271.5.2795)

[64] Banka PA, Behera AP, Sarkar S, Datta AB. 2015 RING E3-Catalyzed E2 Self-Ubiquitination Attenuates the Activity of Ube2E Ubiquitin-Conjugating Enzymes. *J Mol Biol* 427, 2290–2304. (doi:10.1016/j.jmb.2015.04.011)

[65] Kimura M, Hattori T, Matsuda Y, Yoshioka T, Sumi N, Umeda Y, Nakashima S, Okano Y. 1997 cDNA cloning, characterization, and chromosome mapping of UBE2E2 encoding a human ubiquitin-conjugating E2 enzyme. *Cytogenet Genome Res* 78, 107–111. (doi:10.1159/000134639)

[66] Ito K, Kato S, Matsuda Y, Kimura M, Okano Y. 1999 cDNA cloning, characterization, and chromosome mapping of UBE2E3 (alias UbcH9), encoding an N-terminally extended human ubiquitin-conjugating enzyme. *Cytogenet Genome Res* 84, 99–104. (doi:10.1159/000015229)

[67] Plafker SM, Plafker KS, Weissman AM, Macara IG. 2004 Ubiquitin charging of human class III ubiquitin-conjugating enzymes triggers their nuclear import. *J Cell Biology* 167, 649–659. (doi:10.1083/jcb.200406001)

[68] Johnson ES. 2004 PROTEIN MODIFICATION BY SUMO. *Annu Rev Biochem* 73, 355–382. (doi:10.1146/annurev.biochem.73.011303.074118)

[69] Silver ET, Gwozd TJ, Ptak C, Goebel M, Ellison MJ. 1992 A chimeric ubiquitin conjugating enzyme that combines the cell cycle properties of CDC34 (UBC3) and the DNA repair properties of RAD6 (UBC2): implications for the structure, function and evolution of the E2s. *Embo J* 11, 3091–3098.

[70] Sullivan ML, Vierstra RD. 1991 Cloning of a 16-kDa ubiquitin carrier protein from wheat and *Arabidopsis thaliana*. Identification of functional domains by in vitro mutagenesis. *J Biological Chem* 266, 23878–23885.

[71] Ichimura Y *et al.* 2000 A ubiquitin-like system mediates protein lipidation. *Nature* 408, 488–492. (doi:10.1038/35044114)

[72] Huang DT, Miller DW, Mathew R, Cassell R, Holton JM, Roussel MF, Schulman BA. 2004 A unique E1-E2 interaction required for optimal conjugation of the ubiquitin-like protein NEDD8. *Nat Struct Mol Biol* 11, 927–935. (doi:10.1038/nsmb826)

- [73] VanDemark AP, Hill CP. 2004 Grabbing E2 by the tail. *Nat Struct Mol Biol* 11, 908–909. (doi:10.1038/nsmb1004-908)
- [74] Wang Y, Argiles-Castillo D, Kane EI, Zhou A, Spratt DE. 2020 HECT E3 ubiquitin ligases – emerging insights into their biological roles and disease relevance. *J Cell Sci* 133, jcs228072. (doi:10.1242/jcs.228072)
- [75] Huibregtse JM, Scheffner M, Beaudenon S, Howley PM. 1995 A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. *Proc National Acad Sci* 92, 2563–2567. (doi:10.1073/pnas.92.7.2563)
- [76] Berndsen CE, Wolberger C. 2014 New insights into ubiquitin E3 ligase mechanism. *Nat Struct Mol Biol* 21, 301–307. (doi:10.1038/nsmb.2780)
- [77] Zheng N, Shabek N. 2015 Ubiquitin Ligases: Structure, Function, and Regulation. *Annu Rev Biochem* 86, 1–29. (doi:10.1146/annurev-biochem-060815-014922)
- [78] Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. 1990 The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63, 1129–1136. (doi:10.1016/0092-8674(90)90409-8)
- [79] Werness, Levine A, Howley P. 1990 Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science (New York, N.Y.)* 248, 76–9. (doi:10.1126/science.2157286)
- [80] Ingham RJ, Gish G, Pawson T. 2004 The Nedd4 family of E3 ubiquitin ligases: functional diversity within a common modular architecture. *Oncogene* 23, 1972–1984. (doi:10.1038/sj.onc.1207436)
- [81] Bakkers J, Camacho-Carvajal M, Nowak M, Kramer C, Danger B, Hammerschmidt M. 2005 Destabilization of Δ Np63 α ; by Nedd4-Mediated Ubiquitination Ubc9-Mediated Sumoylation, and Its Implications on Dorsoventral Patterning of the Zebrafish Embryo. *Cell Cycle* 4, 790–800. (doi:10.4161/cc.4.6.1694)
- [82] Xu C, Fan CD, Wang X. 2015 Regulation of Mdm2 protein stability and the p53 response by NEDD4-1 E3 ligase. *Oncogene* 34, 281–289. (doi:10.1038/onc.2013.557)
- [83] Li Y, Ozaki T, Kikuchi H, Yamamoto H, Ohira M, Nakagawara A. 2008 A novel HECT-type E3 ubiquitin protein ligase NEDL1 enhances the p53-mediated apoptotic cell death in its catalytic activity-independent manner. *Oncogene* 27, 3700–3709. (doi:10.1038/sj.onc.1211032)
- [84] Miyazaki K, Ozaki T, Kato C, Hanamoto T, Fujita T, Irino S, Watanabe K, Nakagawa T, Nakagawara A. 2003 A novel HECT-type E3 ubiquitin ligase, NEDL2, stabilizes p73 and enhances its transcriptional activity. *Biochem Bioph Res Co* 308, 106–113. (doi:10.1016/s0006-291x(03)01347-0)
- [85] Peschiaroli A, Scialpi F, Bernassola F, Sherbini ESE, Melino G. 2010 The E3 ubiquitin ligase WWP1 regulates Δ Np63-dependent transcription through Lys63 linkages. *Biochem Bioph Res Co* 402, 425–430. (doi:10.1016/j.bbrc.2010.10.050)
- [86] Holloway A, Simmonds M, Azad A, Fox JL, Storey A. 2015 Resistance to UV-induced apoptosis by β -HPV5 E6 involves targeting of activated BAK for proteolysis by recruitment of the HERC1 ubiquitin ligase. *Int J Cancer* 136, 2831–2843. (doi:10.1002/ijc.29350)

- [87] Wu W, Sato K, Koike A, Nishikawa H, Koizumi H, Venkitaraman AR, Ohta T. 2010 HERC2 Is an E3 Ligase That Targets BRCA1 for Degradation. *Cancer Res* 70, 6384–6392. (doi:10.1158/0008-5472.can-10-1304)
- [88] Gao K *et al.* 2015 The E3 ubiquitin ligase Itch and Yap1 have antagonistic roles in the regulation of ASPP2 protein stability. *Febs Lett* 589, 94–101. (doi: 10.1016/j.febslet.2014.11.030)
- [89] Marín I. 2010 Diversification and Specialization of Plant RBR Ubiquitin Ligases. *Plos One* 5, e11579. (doi:10.1371/journal.pone.0011579)
- [90] Fernandez MA *et al.* 2019 RBR-type E3 ligases and the Ub-conjugating enzyme UBC26 regulate ABA receptor levels and signaling. *Plant Physiol* 182, pp.00898.2019. (doi:10.1104/pp.19.00898)
- [91] Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, Chanda SK, Batalov S, Joazeiro CAP. 2008 Genome-Wide and Functional Annotation of Human E3 Ubiquitin Ligases Identifies MULAN, a Mitochondrial E3 that Regulates the Organelle's Dynamics and Signaling. *Plos One* 3, e1487. (doi:10.1371/journal.pone.0001487)
- [92] Freemont PS, Hanson IM, Trowsdale J. 1991 A novel cysteine-rich sequence motif. *Cell* 64, 483–484. (doi: 10.1016/0092-8674(91)90229-r)
- [93] Lovering R *et al.* 1993 Identification and preliminary characterization of a protein motif related to the zinc finger. *Proc National Acad Sci* 90, 2112–2116. (doi:10.1073/pnas.90.6.2112)
- [94] Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM. 1999 RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc National Acad Sci* 96, 11364–11369. (doi:10.1073/pnas.96.20.11364)
- [95] Borden KL, Freemont PS. 1996 The RING finger domain: a recent example of a sequence—structure family. *Curr Opin Struc Biol* 6, 395–401. (doi:10.1016/s0959-440x(96)80060-1)
- [96] Katoh M. 2001 Molecular Cloning and Characterization of RNF26 on Human Chromosome 11q23 Region, Encoding a Novel RING Finger Protein with Leucine Zipper. *Biochem Bioph Res Co* 282, 1038–1044. (doi:10.1006/bbrc.2001.4671)
- [97] Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, Yarden Y. 1998 c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Gene Dev* 12, 3663–3674. (doi:10.1101/gad.12.23.3663)
- [98] Christensen DE, Brzovic PS, Klevit RE. 2007 E2–BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages. *Nature Structural and Molecular Biology* 14, nsmb1295. (doi:10.1038/nsmb1295)
- [99] Christensen DE, Klevit RE. 2009 Dynamic interactions of proteins in complex networks: identifying the complete set of interacting E2s for functional investigation of E3-dependent protein ubiquitination. *FEBS Journal* 276, 5381–5389. (doi:10.1111/j.1742-4658.2009.07249.x)
- [100] Habu T, Harada KH. 2021 UBC13 is an RNF213-associated E2 ubiquitin-conjugating enzyme, and Lysine 63-linked ubiquitination by the RNF213-UBC13 axis is responsible for angiogenic activity. *Faseb Bioadvances* (doi: 10.1096/fba.2019-00092)

- [101] Özkan E, Yu H, Deisenhofer J. 2005 Mechanistic insight into the allosteric activation of a ubiquitin-conjugating enzyme by RING-type ubiquitin ligases. *P Natl Acad Sci Usa* 102, 18890–18895. (doi:10.1073/pnas.0509418102)
- [102] Seol JH *et al.* 1999 Cdc53/cullin and the essential Hrt1 RING–H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. *Gene Dev* 13, 1614–1626. (doi:10.1101/gad.13.12.1614)
- [103] Metzger MB, Pruneda JN, Klevit RE, Weissman AM. 2014 RING-type E3 ligases: Master manipulators of E2 ubiquitin-conjugating enzymes and ubiquitination. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1843, 47–60. (doi:10.1016/j.bbamcr.2013.05.026)
- [104] Linke K, Mace PD, Smith CA, Vaux DL, Silke J, Day CL. 2008 Structure of the MDM2/MDMX RING domain heterodimer reveals dimerization is required for their ubiquitylation in trans. *Cell death and differentiation* 15, 841–848. (doi:10.1038/sj.cdd.4402309)
- [105] Wu-Baer F, Lagrason K, Yuan W, Baer R. 2003 The BRCA1/BARD1 Heterodimer Assembles Polyubiquitin Chains through an Unconventional Linkage Involving Lysine Residue K6 of Ubiquitin. *Journal of Biological Chemistry* 278, 34743–34746. (doi: 10.1074/jbc.C300249200)
- [106] Buchwald G, Stoop P van der, Weichenrieder O, Perrakis A, Lohuizen M van, Sixma TK. 2006 Structure and E3-ligase activity of the Ring-Ring complex of polycomb proteins Bmi1 and Ring1b. *The EMBO journal* 25, 2465–2474. (doi:10.1038/sj.emboj.7601144)
- [107] Yin Q *et al.* 2009 E2 interaction and dimerization in the crystal structure of TRAF6. *Nature structural & molecular biology* 16, 658–666. (doi:10.1038/nsmb.1605)
- [108] Liew CW, Sun H, Hunter T, Day CL. 2010 RING domain dimerization is essential for RNF4 function. *Biochem J* 431, 23–29. (doi: 10.1042/bj20100957)
- [109] Mace PD, Linke K, Feltham R, Schumacher F-R, Smith CA, Vaux DL, Silke J, Day CL. 2008 Structures of the cIAP2 RING Domain Reveal Conformational Changes Associated with Ubiquitin-conjugating Enzyme (E2) Recruitment*. *J Biol Chem* 283, 31633–31640. (doi:10.1074/jbc.m804753200)
- [110] Park YC, Burkitt V, Villa AR, Tong L, Wu H. 1999 Structural basis for self-association and receptor recognition of human TRAF2. *Nature* 398, 533–538. (doi:10.1038/19110)
- [111] Polekhina G, House CM, Traficante N, Mackay JP, Relaix F, Sassoon DA, Parker MW, Bowtell DDL. 2002 Siah ubiquitin ligase is structurally related to TRAF and modulates TNF- α signaling. *Nat Struct Biol* 9, 68–75. (doi: 10.1038/nsb743)
- [112] Dou H, Buetow L, Sibbet GJ, Cameron K, Huang DT. 2012 BIRC7–E2 ubiquitin conjugate structure reveals the mechanism of ubiquitin transfer by a RING dimer. *Nat Struct Mol Biol* 19, 876–883. (doi:10.1038/nsmb.2379)
- [113] Koepp DM, Harper JW, Elledge SJ. 1999 How the Cyclin Became a Cyclin Regulated Proteolysis in the Cell Cycle. *Cell* 97, 431–434. (doi:10.1016/s0092-8674(00)80753-9)
- [114] Murray A. 1995 Cyclin Ubiquitination: The destructive end of mitosis. *Cell* 81, 149–152. (doi:10.1016/0092-8674(95)90322-4)

- [115] King RW, Deshaies RJ, Peters J-M, Kirschner MW. 1996 How Proteolysis Drives the Cell Cycle. *Science* 274, 1652–1659. (doi:10.1126/science.274.5293.1652)
- [116] Deshaies RJ. 1999 SCF AND CULLIN/RING H2-BASED UBIQUITIN LIGASES. *Annu Rev Cell Dev Bi* 15, 435–467. (doi:10.1146/annurev.cellbio.15.1.435)
- [117] Kipreos ET, Pagano M. 2000 The F-box protein family. *Genome Biol* 1, reviews3002.1. (doi:10.1186/gb-2000-1-5-reviews3002)
- [118] Nakayama KI, Nakayama K. 2006 Ubiquitin ligases: cell-cycle control and cancer. *Nat Rev Cancer* 6, 369–381. (doi:10.1038/nrc1881)
- [119] Cope GA, Deshaies RJ. 2003 COP9 Signalosome A Multifunctional Regulator of SCF and Other Cullin-Based Ubiquitin Ligases. *Cell* 114, 663–671. (doi:10.1016/s0092-8674(03)00722-0)
- [120] Gmachl M, Gieffers C, Podtelejnikov AV, Mann M, Peters J-M. 2000 The RING-H2 finger protein APC11 and the E2 enzyme UBC4 are sufficient to ubiquitinate substrates of the anaphase-promoting complex. *Proc National Acad Sci* 97, 8973–8978. (doi:10.1073/pnas.97.16.8973)
- [121] Zachariae W, Shevchenko A, Andrews PD, Ciosk R, Galova M, Stark MJR, Mann M, Nasmyth K. 1998 Mass Spectrometric Analysis of the Anaphase-Promoting Complex from Yeast: Identification of a Subunit Related to Cullins. *Science* 279, 1216–1219. (doi:10.1126/science.279.5354.1216)
- [122] Leversen JD, Joazeiro CAP, Page AM, Huang H, Hieter P, Hunter T. 2000 The APC11 RING-H2 Finger Mediates E2-Dependent Ubiquitination. *Mol Biol Cell* 11, 2315–2325. (doi:10.1091/mbc.11.7.2315)
- [123] Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters J-M. 2000 Mitotic Regulation of the APC Activator Proteins CDC20 and CDH1. *Mol Biol Cell* 11, 1555–1569. (doi:10.1091/mbc.11.5.1555)
- [124] Visintin R, Prinz S, Amon A. 1997 CDC20 and CDH1: A Family of Substrate-Specific Activators of APC-Dependent Proteolysis. *Science* 278, 460–463. (doi:10.1126/science.278.5337.460)
- [125] Vodermaier HC, Gieffers C, Maurer-Stroh S, Eisenhaber F, Peters J-M. 2003 TPR Subunits of the Anaphase-Promoting Complex Mediate Binding to the Activator Protein CDH1. *Curr Biol* 13, 1459–1468. (doi:10.1016/s0960-9822(03)00581-5)
- [126] Kramer ER, Gieffers C, Hölzl G, Hengstschräger M, Peters J-M. 1998 Activation of the human anaphase-promoting complex by proteins of the CDC20/Fizzy family. *Curr Biol* 8, 1207–1254. (doi:10.1016/s0960-9822(07)00510-6)
- [127] Raff JW, Jeffers K, Huang J. 2002 The roles of Fzy/Cdc20 and Fzr/Cdh1 in regulating the destruction of cyclin B in space and time. *J Cell Biology* 157, 1139–1149. (doi:10.1083/jcb.200203035)
- [128] Prinz S, Hwang ES, Visintin R, Amon A. 1998 The regulation of Cdc20 proteolysis reveals a role for the APC components Cdc23 and Cdc27 during S phase and early mitosis. *Curr Biol* 8, 750–760. (doi:10.1016/s0960-9822(98)70298-2)
- [129] Passmore LA, McCormack EA, Au SWN, Paul A, Willison KR, Harper JW, Barford D. 2003 Doc1

mediates the activity of the anaphase-promoting complex by contributing to substrate recognition. *Embo J* 22, 786–796. (doi:10.1093/emboj/cdg084)

[130] Au SWN, Leng X, Harper JW, Barford D. 2002 Implications for the Ubiquitination Reaction of the Anaphase-promoting Complex from the Crystal Structure of the Doc1/Apc10 Subunit. *J Mol Biol* 316, 955–968. (doi: 10.1006/jmbi.2002.5399)

[131] Carroll CW, Morgan DO. 2002 The Doc1 subunit is a processivity factor for the anaphase-promoting complex. *Nat Cell Biol* 4, 880–887. (doi:10.1038/ncb871)

[132] Pflieger CM, Lee E, Kirschner MW. 2001 Substrate recognition by the Cdc20 and Cdh1 components of the anaphase-promoting complex. *Gene Dev* 15, 2396–2407. (doi:10.1101/gad.918201)

[133] Burton JL, Solomon MJ. 2001 D box and KEN box motifs in budding yeast Hsl1p are required for APC-mediated degradation and direct binding to Cdc20p and Cdh1p. *Gene Dev* 15, 2381–2395. (doi:10.1101/gad.917901)

[134] Kraft C, Vodermaier HC, Maurer-Stroh S, Eisenhaber F, Peters J-M. 2005 The WD40 Propeller Domain of Cdh1 Functions as a Destruction Box Receptor for APC/C Substrates. *Mol Cell* 18, 543–553. (doi:10.1016/j.molcel.2005.04.023)

[135] Tian W, Li B, Warrington R, Tomchick DR, Yu H, Luo X. 2012 Structural analysis of human Cdc20 supports multisite degron recognition by APC/C. *Proc National Acad Sci* 109, 18419–18424. (doi:10.1073/pnas.1213438109)

[136] Zur A, Brandeis M. 2002 Timing of APC/C substrate degradation is determined by fzy/fzr specificity of

destruction boxes. *Embo J* 21, 4500–4510. (doi:10.1093/emboj/cdf452)

[137] Glotzer M, Murray AW, Kirschner MW. 1991 Cyclin is degraded by the ubiquitin pathway. *Nature* 349, 132–138. (doi:10.1038/349132a0)

[138] Ayad NG, Rankin S, Ooi D, Rape M, Kirschner MW. 2005 Identification of Ubiquitin Ligase Substrates by In Vitro Expression Cloning. *Methods Enzymol* 399, 404–414. (doi:10.1016/s0076-6879(05)99028-9)

[139] Pflieger CM, Kirschner MW. 2000 The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. *Gene Dev* 14, 655–665.

[140] Reis A, Levasseur M, Chang H, Elliott DJ, Jones KT. 2006 The CRY box: a second APCcdh1-dependent degron in mammalian cdc20. *Embo Rep* 7, 1040–1045. (doi:10.1038/sj.embor.7400772)

[141] Zachariae W, Schwab M, Nasmyth K, Seufert W. 1998 Control of Cyclin Ubiquitination by CDK-Regulated Binding of Hct1 to the Anaphase Promoting Complex. *Science* 282, 1721–1724. (doi:10.1126/science.282.5394.1721)

[142] Harper JW, Burton JL, Solomon MJ. 2002 The anaphase-promoting complex: it's not just for mitosis any more. *Gene Dev* 16, 2179–2206. (doi:10.1101/gad.1013102)

[143] Yu H. 2007 Cdc20: A WD40 Activator for a Cell Cycle Degradation Machine. *Mol Cell* 27, 3–16. (doi: 10.1016/j.molcel.2007.06.009)

[144] Baker DJ, Dawlaty MM, Galardy P, Deursen JM van. 2007 Mitotic regulation of the anaphase-promoting complex. *Cell Mol Life Sci* 64, 589–600. (doi: 10.1007/s00018-007-6443-1)

[145] Aravind L, Koonin EV. 2000 The U box is a modified RING finger — a common domain in ubiquitination. *Curr Biol* 10, R132–R134. (doi:10.1016/s0960-9822(00)00398-5)

[146] Hatakeyama S, Yada M, Matsumoto M, Ishida N, Nakayama K-I. 2001 U Box Proteins as a New Family of Ubiquitin-Protein Ligases. *J Biol Chem* 276, 33111–33120. (doi:10.1074/jbc.m102755200)

[147] Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin L-Y, Patterson C. 1999 Identification of CHIP, a Novel Tetratricopeptide Repeat-Containing Protein That Interacts with Heat Shock Proteins and Negatively Regulates Chaperone Functions. *Mol Cell Biol* 19, 4535–4545. (doi:10.1128/mcb.19.6.4535)

[148] Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Höhfeld J, Patterson C. 2001 CHIP Is a U-box-dependent E3 Ubiquitin Ligase IDENTIFICATION OF Hsc70 AS A TARGET FOR UBIQUITYLATION. *J Biol Chem* 276, 42938–42944. (doi:10.1074/jbc.m101968200)

[149] Hatakeyama S, Nakayama KI. 2003 U-box proteins as a new family of ubiquitin ligases. *Biochem Biophys Res Commun* 302, 635–645. (doi:10.1016/s0006-291x(03)00245-6)