Critical Reviews in Clinical Laboratory Sciences

http://informahealthcare.com/lab
ISSN: 1040-8363 (print), 1549-781X (electronic)

Crit Rev Clin Lab Sci, 2014; 51(5): 280–290
 DOI: 10.3109/10408363.2014.915287

informa healthcare

REVIEW ARTICLE

The role of ubiquitin-binding domains in human pathophysiology

Kleitos Sokratous[1], Andreas Hadjisavvas[1,2], Eleftherios P. Diamandis[3,4], and Kyriacos Kyriacou[1,2]

[1]The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [2]Cyprus School of Molecular Medicine, Nicosia, Cyprus, [3]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada, and [4]Department of Clinical Biochemistry, University Health Network, Toronto, Ontario, Canada

Abstract

Ubiquitination, a fundamental post-translational modification (PTM) resulting in the covalent attachment of ubiquitin (Ub) to a target protein, is currently implicated in several key cellular processes. Although ubiquitination was initially associated with protein degradation, it is becoming increasingly evident that proteins labeled with polyUb chains of specific topology and length are activated in an ever-expanding repertoire of specific cellular processes. In addition to their involvement in the classical protein degradation pathways they are involved in DNA repair, kinase regulation and nuclear factor-κB (NF-κB) signaling. The sorting and processing of distinct Ub signals is mediated by small protein motifs, known as Ub-binding domains (UBDs), which are found in proteins that execute disparate biological functions. The involvement of UBDs in several biological pathways has been revealed by several studies which have highlighted the vital role of UBDs in cellular homeostasis. Importantly, functional impairment of UBDs in key regulatory pathways has been related to the development of pathophysiological conditions, including immune disorders and cancer. In this review, we present an up-to-date account of the crucial role of UBDs and their functions, with a special emphasis on their functional impairment in key biological pathways and the pathogenesis of several human diseases. The still under-investigated topic of Ub-UBD interactions as a target for developing novel therapeutic strategies against many diseases is also discussed.

Abbreviations: AD: Alzheimer's disease; **Aβ**: amyloid-β peptide; **BARD**: BRCA1-associated RING domain protein; **BRCA1**: breast cancer susceptibility protein 1; **CUE**: coupling of ubiquitin conjugation to endoplasmic reticulum degradation motif; **DUB**: deubiquitinase; **EPS15**: epidermal growth factor receptor pathway substrate 15; **FA**: Fanconi anemia; **FANCD2**: Fanconi anemia complementation group D2 protein; **FANCI**: Fanconi anemia complementation group I protein; **hHR23A**: human homologue Rad23A; **IAP**: inhibitor of apoptosis; **IsoT**: isopeptidase-T; **LUBAC**: linear ubiquitin assembly complex; **LUBID**: linear ubiquitin-binding domain; **MDM2**: murine double minute oncogene; **MIU**: motif interacting with ubiquitin; **MVB**: multivesicular bodies; **NEMO**: NF-κB essential modulator; **NF-κB**: nuclear factor-kappa B; **NOA**: NEMO Optineurin ABIN; **PCNA**: proliferating cell nuclear antigen; **PD**: Paget's disease of bone; **PTM**: post-translational modification; **RAP80**: receptor-associated protein 80; **RUZ**: Rabex-5 ubiquitin-binding ZnF; **TC-NER**: transcription-coupled nucleotide excision repair; **TLS**: translation synthesis; **Ub**: ubiquitin; **UBA**: ubiquitin-associated; **UBAN**: ubiquitin binding in ABIN and NEMO; **UBD**: ubiquitin-binding domain; **UBI**: ubiquitin-binding inhibitor; **UBZ**: ubiquitin-binding zinc finger; **UIM**: ubiquitin-interacting motif; **UQ1**: ubiquilin-1; **UPS**: ubiquitin-proteasome system; **USP**: ubiquitin-specific protease; **ZnF**: zinc finger; **Clastogenic**: capable of causing chromosomal breakages; **Oncogenic**: causing development of a tumor.

Keywords

Ubiquitin, UBD, ubiquitination, signaling pathways, immunodeficiency disorders, tumorigenesis

History

Received 17 January 2014
Revised 8 April 2014
Accepted 11 April 2014
Published online 5 June 2014

Introduction

The functional diversity and dynamics of the eukaryotic proteome is mainly attributed to post-translational modifications (PTMs) of proteins such as phosphorylation, methylation, acetylation and ubiquitination. The first three modifications involve the addition of a small chemical group (phosphate, methyl and acetyl groups, respectively), whereas the latter entails the conjugation of a small protein,

Referees: Dr. David Fushman, Department of Chemistry & Biochemistry, University of Maryland College Park, MD, USA; Dr. Gregory Tsongalis, Translational Research Core Facility, Department of Pathology, Dartmouth Medical School, Lebanon, NH, USA

Address for correspondence: Kyriacos Kyriacou, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus. E-mail: kyriacos@cing.ac.cy

namely Ub, to a target protein. Ubiquitination was initially recognized as the trigger for protein degradation through the Ub-proteasome proteolytic pathway. Currently, almost 40 years after its discovery, ubiquitination constitutes a fundamental regulatory mechanism involved in several key eukaryotic cellular processes (reviewed in references[1–6]), including protein degradation[7], vesicular trafficking[8], DNA repair[9], endocytosis[10] and transcription and gene silencing[11].

Crucial to the function of Ub as a signaling motif is its non-covalent association with modular protein domains known as UBDs. These motifs are found in proteins known as Ub receptor proteins, which execute disparate biological functions; for example, UBDs are present in the degradation machinery proteasome 26S and in the endocytic machinery EPS15 (epidermal growth factor receptor pathway substrate 15), which function in all eukaryotes. In addition, UBDs are present in E2-conjugating enzymes[11–13]. Therefore, this non-covalent interaction between Ub and UBDs, may promote the covalent attachment of Ub to a target protein and the formation of polyUb chains of specific length and topology, as well as direct the fate of ubiquitinated proteins leading to regulation of many cellular processes. The latter include proteasome degradation, endocytosis, autophagy, DNA repair and damage tolerance, multivesicular bodies biogenesis and NF-κB signaling. Accumulated knowledge about the functional and structural diversity of UBDs and their role in modifying protein function has been recently summarized in a review by Dikic et al.[13]

The aim of this review is to present a critical account of the current knowledge on the involvement of UBDs in the pathogenesis of several human diseases such as cancer and immunodeficiency disorders. In addition, special emphasis will be devoted to discussing the different classes of UBDs, the importance of the non-covalent Ub-UBD interactions in multiple cellular processes as well as the consequences of their dysregulation. Most of the accumulated knowledge that highlights the role of UBDs in human disorders is discussed in terms of their functional impairment in key cellular pathways, such as the DNA repair and the DNA damage response pathways. In this context, an up-to-date account of the role of UBDs in the pathogenesis of several human diseases, including cancer and immunodeficiency disorders, will be presented and discussed in relation to the respective pathways that are dysregulated mainly due to alterations in UBDs, which subsequently give rise to these disorders. Moreover, the still under-investigated topic of Ub-UBD interactions as targets for developing novel therapeutic strategies against many diseases will be highlighted.

Ubiquitin and ubiquitination

Ub is a 76–residue protein (~8.5 kDa) that is ubiquitously expressed in eukaryotic organisms and is highly conserved among the eukaryotes. Its secondary structure is defined by a 3.5-turn α-helix, a 3_{10} helix and a 5-stranded β-sheet (Figure 1a). The human and yeast Ub share a 96% sequence homology, with 73 out of 76 amino acids located at the same position[1], which leads to the conclusion that the primary structure, and thus the secondary structure of Ub, are essential for its biological functionality. Indeed, key functional features of the Ub protein include its C-terminal and the seven lysine (Lys) residues, located at positions 6, 11, 27, 29, 33, 48 and 63, which permit the formation of polyUb chains. These Ub multimers can be both homogeneously linked (i.e. moieties having the same linkage throughout the chain, for example, Lys48- or Lys63-linked) and mixed-linked (i.e. chains containing different Lys linkages, for example, a mixture of Lys48- and Lys63-linked). The structure, assembly and function of Ub as a signaling motif have been recently reviewed by Komander and Rape[14].

Ubiquitination is catalyzed by a triplet of enzymes, namely E1, E2 and E3, and leads to the formation of an isopeptide bond between the C-terminal glycine (Gly) carboxyl group of Ub and, most often, a Lys residue in the target protein (Figure 2). However, covalent attachment of Ub to a target protein through the substrate's N-terminus[15], the thiol group of cysteine (Cys) (formation of thioester bond)[16] and the hydroxyl group of serine (Ser) or threonine (Thr) residues[17] have also been reported. The ubiquitination cycle starts with the activation of the C-terminus of Ub by an E1 activating enzyme. The activated Ub moiety is then attached to a Cys residue of an E2-conjugating enzyme, and finally transferred to an amino group (usually ε-amino group of a Lys residue) of the substrate through the E3 Ub protein ligase enzyme. The ligation of Ub to the target protein can be catalyzed via two pathways (herein referred to as pathways A and B), depending on the type of E3 enzyme that participates (Figure 2). Particularly, in pathway A the HECT-domain E3s form a thioester bond with Ub prior to substrate attachment. In contrast, in pathway B the RING or U-box domain E3s, instead of forming a straight interaction with Ub, function as a bridge between an activated E2 and the substrate[14,18].

Repetition of the ubiquitination cycle leads to the formation of polyUb chains by utilizing any of the seven Lys residues of the Ub monomer, as well as Ub's N-terminus (linear polyUb chains). Strong evidence is emerging that proteins labeled with polyUb chains of a specific topology and length are channeled to a specific cellular process

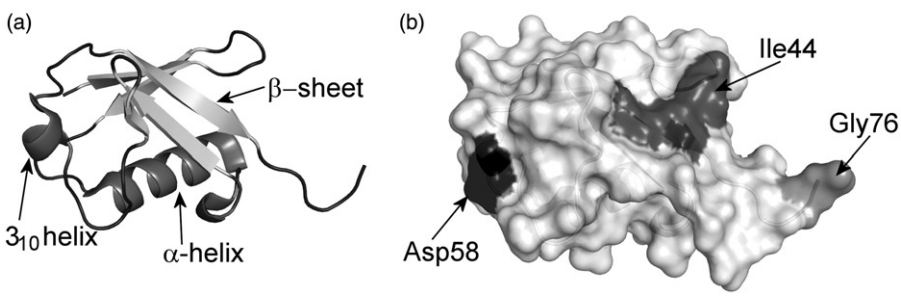

Figure 1. Cartoon and surface representations of Ub. (a) The secondary structure of Ub is defined by a 3.5-turn α-helix, a 3_{10} helix and a 5-stranded β-sheet. (b) Surface representation of Ub showing the Ile44 hydrophobic patch, the polar patch Asp58 and the C-terminal Gly76 residue. Protein Data Bank (PDB) identifier (ID): 1D3Z[151].

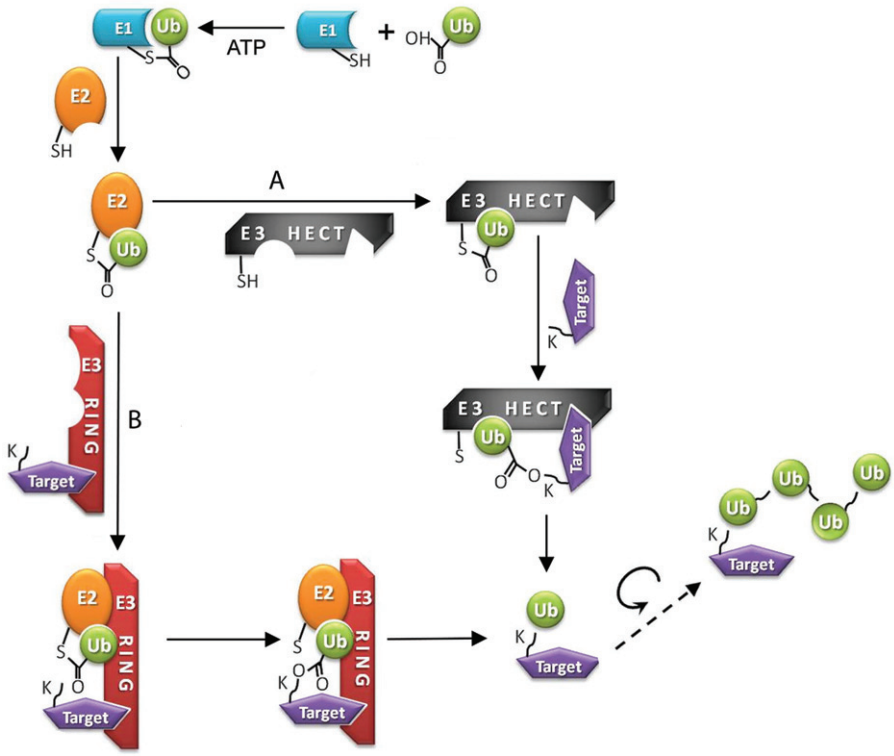

Figure 2. The ubiquitination pathway. The C-terminus of a Ub moiety is activated by an E1 enzyme via an ATP-dependent step that initially forms a Ub-adenylate intermediate (not shown), which leads to the formation of a thioester bond between a side chain of a Cys residue of an E1 enzyme and the C-terminus of Ub. The activated Ub moiety is then attached to a Cys residue of an E2-conjugating enzyme. Ligation of Ub to the target protein can be catalyzed by either the HECT-domain E3s (pathway A), which form a thioester bond with Ub prior substrate attachment, or by the RING-domain E3s or U-box E3s (pathway B), which functions as a bridge between an activated E2 and the substrate.

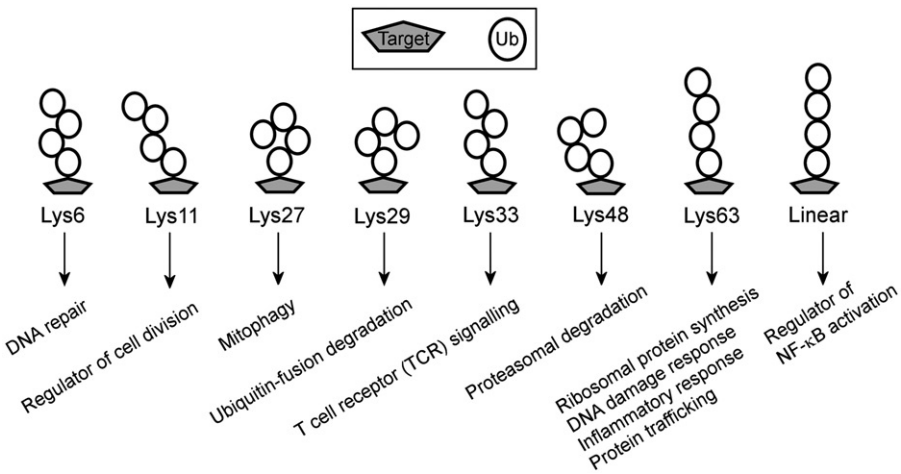

Figure 3. Cellular pathways associated with polyUb chains of a specific topology.

(Figure 3). For example, Lys48-linked chains classically signal the target protein for degradation by the proteasome[19], whereas Lys63-linked chains act as an activation trigger in a variety of pathways, including DNA damage tolerance[20], the inflammatory response[21], protein trafficking[22] and regulation of protein synthesis[23]. In addition, it has been reported that Lys11-linked chains serve as potent proteasomal degradation signals[24]. Studies on the BRCA1-BARD (breast cancer susceptibility protein 1 – BRCA1-associated RING domain protein) complex have shown that Lys6-linked polyUb chains are likely to be involved in DNA repair[25,26]. Moreover, the linear polyUb chains also known as head-to-tail, which are assembled by a specific ligase complex named linear Ub chain assembly complex (LUBAC)[27,28], are critical for NF-κB signaling[28–30]. A recent study by Nakasone et al. also revealed that mixed-linkage tri-Ub chains, containing both Lys48 and Lys63 linkages, preserve the signaling properties of each linkage[31]. The intrinsic importance and functions of polyUb chains of diverse linkage are extensively discussed in other recently published reviews[32–34].

Role of UBDs in the ubiquitin pathway

Recognition of discrete Ub signals and their subsequent channeling to specific cellular processes is mediated by non-covalent interactions of Ub (or ubiquitinated substrates) with modular protein domains known as UBDs. Several UBDs have been identified and categorized in more than 20 families. Members of the same family show structural similarities and it is believed that they interact with the same region of Ub to form a non-covalent Ub-UBD complex (reviewed in references[11–13]). For instance, Ub-associated (UBA) domains belong to the family of α-helical UBDs and are known to non-covalently bind Ub via a hydrophobic patch located in the β-sheet region of Ub, known as the Ile44 hydrophobic patch (Figures 1b and 4a). Examples of these

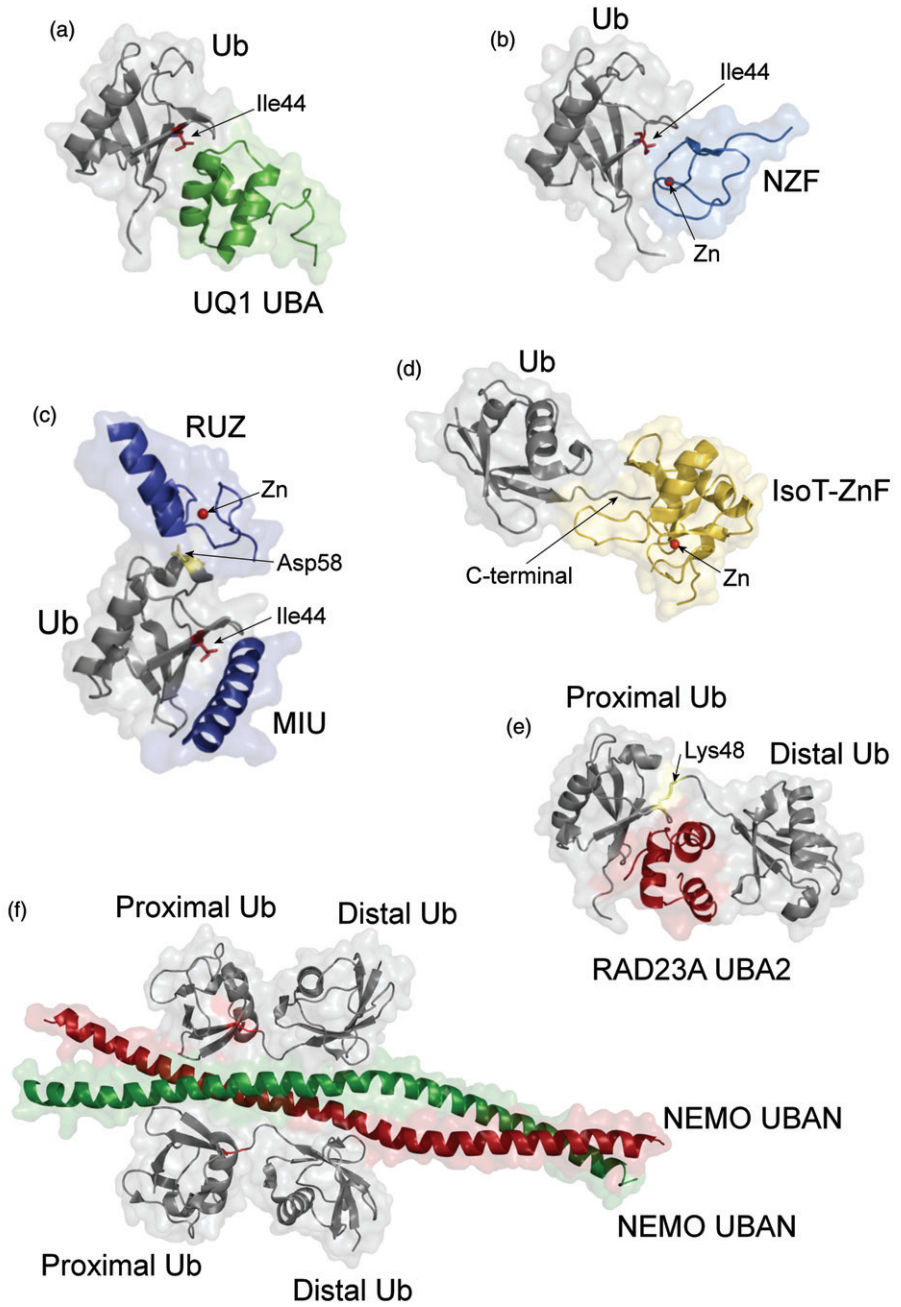

Figure 4. The structures of several Ub-UBD complexes. (a) Cartoon representation of Ub in complex with the triple-helix bundle UBA domain of UQ1 (PDB ID: 2JY6)[62]. (b) Cartoon representation of Ub in complex with the NZF domain (PDB ID: 1Q5W)[52]. (c) Cartoon representation of Ub in complex with the RUZ and MIU domains of Rabex-5. The RUZ domain binds to an Asp58-centered surface and the single-helix domain, MIU, binds to the Ile44 hydrophobic patch (PDB ID: 2FIF)[41]. (d) Cartoon representation of Ub in complex with the ZnF domain of IsoT. The domain binds to the carboxyl terminus of Ub. (e) Cartoon representation of Lys48-linked diUb in complex with the UBA2 domain of RAD23A (PDB ID: 1ZO6)[60]. (f) The UBAN domain in NEMO forms a coiled coil, which binds two linear diUb (PDB ID: 2ZVO)[30].

include the UBA domain of ubiquilin-1 (UQ1), a protein that acts as a presenilin regulator[35], and the two UBA domains (UBA1 and UBA2) of the human homologue Rad23A (hHR23A), a protein that mediates the delivery of substrates to the 26S proteasome[36], the regulation of p53[37] and nucleotide excision repair[38]. Similar to the three-helix bundle UBA domains, the single α-helix ubiquitin-interacting motif (UIM) domains, including motif interacting with Ub (MIU) and double-sided UIM, also bind Ub via Ub's Ile44 hydrophobic patch[39–42]. Nevertheless, a recent study showed that point mutations in Ub's hydrophobic core can cause specific effects upon functions of Ub[43]. Particularly, it was shown that the point mutations Leu67Ser and Leu69Ser, located in Ub's buried core, disturb interactions of Ub with the UIM domain of the proteasomal Ub receptors S5a and Rpn10, whereas interactions with the UBA domain of the receptor proteins Rad23 and hHR23A remain unimpaired.

In contrast to α-helical domains, zinc finger (ZnF) domains bind to Ub via three different regions (Asp58, Ile44 and Gly76) located on the surface of Ub (Figure 1b). For example, association of the vesicular trafficking protein Rabex-5 with Ub is mediated by a Rabex-5 Ub-binding ZnF (RUZ) domain (Figure 4c), which interacts with an Asp58-centered region and by a MIU domain that binds the Ile44-centered patch on Ub[44]. Similarly, two other members of the ZnF family, the nuclear protein localization 4 ZnF (NZF) domain and the Ub-binding ZnF (UBZ) domain, bind to Ub via its hydrophobic surface around Ile44 (Figure 4b).

UBDs are also present in E2 Ub-conjugating enzymes (UBC), which promote the assembly of polyUb chains of specific linkage topology. For example, it is known that the UBC13/MMS2 heterodimer mediates the assembly of Lys63-linked polyUb chains[45], whereas the UBE2S catalyzes the formation of Lys11-linked polyUb chains[46]. A recent study

suggested that the synthesis of Lys48-linked polyUb chains, which is carried out by the E2-25K enzyme[47], is directed by the C-terminal UBA domain of E2-25K[48]. Interestingly, it was shown that E2-25K is a crucial mediator of amyloid-β peptide (Aβ) neurotoxicity[49], which is linked to the pathogenesis of Alzheimer's disease (AD)[50]. In addition, the frameshift mutant of Ub B (UBB^{+1}) is a potent inhibitor of the Ub-proteasome system (UPS) that is often observed in AD patients[51–53]. Recent structural data showed that the UBB^{+1} interacts with the UBA domain of E2-25K and forms a complex that promotes the accumulation of UBB^{+1}-anchored polyUbs, which in turn leads to proteasomal inhibition and neurotoxicity[54]. Furthermore, aggregation of extended polyglutamine proteins that result in proteasomal inhibition and development of neurotoxicity in Huntington's disease has been associated with interactions of the E2-25K UBA domain with huntingtin[55].

The deubiquitinating (DUB) enzyme isopeptidase-T (IsoT or USP5) carries out the dissociation of free polyUb chains into monoUb moieties. Since this interaction is mediated by a ZnF Ub-protease (UBP) domain, which recognizes the C-terminal Gly residue of Ub[56], it is believed that the ZnF UBP domain of IsoT has no preference for polyUb chain length or topology (Figure 4d). Indeed, a recent mass spectrometry study has shown that ZnF UBP binds mono-Ub and di-Ub modules with similar affinity[57]. The specific recognition of polyUb chain length and topology by UBDs is only now beginning to emerge. For instance, it has been shown that the hHR23A-UBA domains show a clear selectivity for Lys48-linked (Figure 4e) over Lys11-, Lys27-, Lys29- and Lys63-linked polyUb chains[36,57–61]. In contrast, the UQ1-UBA domain does not possess any binding preference for polyUb chain linkage[57,59,61,62]. It has been suggested that UBDs that interact with polyUb chains in a linkage-independent manner may be particularly useful for capturing the totality of ubiquitinated proteins from complex protein mixtures[59]. Accumulated knowledge about UBDs' preferential binding to polyUb chains of diverse linkage has been summarized in a recent review by Husnjak and Dikic[33].

Role of UBDs in human pathophysiology

The intrinsic importance of UBDs is being increasingly recognized as they have been associated with a variety of pathological conditions, including cancer and immunodeficiency disorders (Table 1). As described above, the range of disorders in which dysregulation of UBDs plays a major role in their pathogenesis will be discussed in terms of the respective pathways in which UBDs are mechanistically involved. For example, functional impairment of UBDs in the NF-κB pathway leads to the development of several disorders, including B-cell lymphomas, autoimmune diseases and Paget's disease of bone (PD). In this context, the role of UBDs in the pathogenesis of human diseases will be discussed in relation to their functional impairment in the following five pathways:

- *UBDs and the NF-κB pathway,*
- *UBDs and the DNA damage response pathway,*
- *UBDs and the nucleotide excision repair pathway,*
- *UBDs and the Fanconi anemia (FA) pathway and*
- *UBDs and the translation synthesis (TLS) pathway.*

UBDs and the NF-κB pathway

NF-κB, a transcription factor formed by Rel proteins, is involved in vital cellular processes, including gene expression, skin homeostasis and immunity. In the canonical pathway, NF-κB factors are retained in an inactive state by binding to the inhibitor of NF-κB, IκB. Upon cell stimulation, IκB is sequentially phosphorylated by the IκB kinase complex IKK, ubiquitinated and finally directed to the proteasome for degradation. The IKK complex consists of two kinases, IKKα and IKKβ, and the regulatory component NF-κB essential modulator (NEMO) also known as IKKγ. This complex is activated by an upstream kinase known as TAK1, which is activated in response to tumor necrosis factor-α (TNF-α) or IL-1 receptor stimulation[63].

Several studies have reported the presence of a UBD in NEMO, commonly referred to as Ub binding in ABIN and NEMO (UBAN), which was initially thought to preferably bind to Lys63-linked polyUb chains[64–67]. A more recent study, however, has shown that NEMO demonstrates significantly higher binding affinity towards linear polyUb chains compared with Lys63-linked chains[68]. Another study has provided structural and functional evidence about the preferential binding of UBAN to linear polyUb chains (Figure 4f), revealing the key role of the UBAN motif of NEMO in the activation of the canonical NF-κB pathway[30]. In particular, it was shown that the UBAN motif binds linear polyUb chains, mainly via hydrophobic interactions with Ub's hydrophobic patch residue Ile44, in addition to the formation of salt bridges and hydrogen bonds. Previous studies showed that the development of X-linked anhidrotic ectodermal dysplasia with immune deficiency syndrome is related to Asp311Asn,

Table 1. Impaired signaling pathways and resulting pathologies related to UBDs.

UBD	Protein	Related pathway	Related pathophysiology	References
UBA	E2-25K	UPS	Alzheimer's and Huntington neurodegenerative diseases	54,55
UBAN	NEMO	NF-κB	X-linked anhidrotic ectodermal dysplasia with immunodeficiency	30,69,70
ZF7	A20	NF-κB	B cell lymphoma	85–87
UBA	IAP	NF-κB	Tumorigenesis	89
UBA	P62	NF-κB	PD	90–94
UIM	RAP80	DNA damage response	Tumorigenesis	102
UMI	RNF168	DNA damage response	Tumorigenesis	108,109
UBD	CSB	TC-NER	Cockayne Syndrome B	113
CUE	FANCD2	FA	Bone marrow failure; cancer susceptibility	127
UBM	TLS polymerases	TLS	Increased UV sensitivity	134,135
UBZ	TLS polymerases	TLS	Increased UV sensitivity	134,135

Glu315Ala and Arg319Gln *NEMO* alleles[69,70]. According to Rahighi et al. any of these mutants will attenuate the ability of UBAN (thus NEMO) to recognize and bind linear Ub chains, which will consequently inhibit the activation of NF-κB pathway[30].

The Ub-editing protein A20 modulates NF-κB activation through a variety of cell surface receptors such as TNF receptors[71], toll-like receptors[72], CD40[73], as well as viral proteins, e.g. the Epstein-Barr virus latent membrane protein 1 (EBV LMP1)[74]. Recent genetic studies have revealed putative associations of polymorphic *A20* (also known as *TNFAIP3*) alleles with systemic lupus erythematosus[75,76]. The vital role of A20 has also been highlighted by recent studies which showed a linkage between A20-deficiency and the development of pathophysiological conditions, including IBD (inflammatory bowel disease) associated arthritis[77], experimental colitis[78], rheumatoid arthritis[79] and autoimmune disorders[80,81]. A study by Shembade et al. showed that A20 can inhibit the E3 ligase activities of TNF receptor-associated factor 6 (TRAF6), TNF receptor-associated factor 2 (TRAF2) and cIAP by antagonizing interactions with the E2 Ub-conjugating enzymes Ubc13 and UbcH5c. In this way, ubiquitination of signaling proteins can be indirectly inhibited by A20[82].

The regulatory activity of A20 is mainly attributed to its Ub-editing functions carried out by UBDs. The N-terminus of A20 contains an ovarian tumor (OTU) UBD domain that specifically recognizes Lys63-linked polyubiquitinated substrates, whereas the C-terminus consists of seven ZnF domains that confer E3 ligase activity to A20[83]. Particularly, A20 cleaves Lys63-linked polyUb chains on receptor-interacting protein 1 (RIP1) and conjugates Lys48-linked polyUb chains, thus targeting RIP1 for proteasomal degradation[83]. Interestingly, it has been shown that mutations in ZnF domains of A20 (hereinafter referred to as ZnF-A20), particularly in ZnF4-A20 and ZnF7-A20, decrease the E3 Ub ligase activity of A20 and also diminish the recruitment of A20 to NEMO following TNF stimulation[84]. Recently, Verhelst et al. have demonstrated the key role of the ZnF7-A20 domain in the negative regulation of NF-κB activation by A20[85]. The study showed that the ZnF7-A20 domain preferentially binds linear polyUb chains *in vitro*, identifying it as a linear Ub-binding domain. Therefore, A20 inhibits LUBAC-induced NF-κB activation by establishing interactions with the LUBAC complex mediated by its ZnF7-A20 domain. Importantly, the study proposed a significant physiological role of the ZnF7-A20 domain in NF-κB suppression based on previous studies which have identified A20 mutants lacking the ZnF7-A20 domain in B cell lymphomas[86,87]. In addition, the authors implied a potential therapeutic use of ZnF7-A20 polypeptides or peptidomimetics against B cell lymphoma and autoimmune diseases[85].

Several studies have shown that the inhibitor of apoptosis proteins (IAPs) are often overexpressed in cancer, and that the levels of IAPs are implicated in contributing to tumorigenesis, chemo-resistance, disease progression and poor patient survival[88]. A recent study has revealed the presence of a UBA domain in IAP that promotes preferable interactions with Lys63-linked polyUb chains[89]. The authors concluded that the UBA domain is essential for the oncogenic potential of cIAP, to maintain endothelial cell survival and to protect cells from TNF-α-induced apoptosis. In addition, according to the study, the UBA domain can modulate NF-κB signaling by binding to polyubiquitinated NEMO or RIP1[89].

PD is a rare, chronic skeletal disorder that causes abnormal bone growth. Several studies have linked mutations affecting the scaffold protein p62/SQSTM1 with the development of PD. It has also been shown that a number of mutations are clustered within the UBA domain of p62[90–93]. Although the precise functional implications of p62/SQSTM1 in the pathogenesis and progression of PD remain to be revealed, it is apparent that p62/SQSTM1 is involved in the regulation of ubiquitinated protein turnover and in the activation of NF-κB. It is believed that mutations within the UBA domain of p62 inhibit p62 ubiquitination which may result in the dysregulation of TRAF6 ubiquitination and downstream NF-κB signaling. Furthermore, a recent structural and biophysical study suggested that the common p62 mutations, Pro392Leu, Ser399Pro, Met404Val/Thr, Gly411Ser and Gly425Arg, which are found in PD patients and are located within the C-terminal UBA domain of p62, hinder p62 ubiquitination via a complex mechanism involving the full-length protein rather than the isolated UBA domain of p62[94]. Recent advances in the understanding of the molecular basis of PD, with respect to the functions of p62/SQSTM1 protein, have been recently reviewed by Goode and Layfield[95] and by Rea et al.[96].

UBDs and the DNA damage response pathway

Recently, it has become apparent that proteins involved in the DNA repair pathway are ubiquitinated in response to DNA damage. Examples include the proliferating cell nuclear antigen (PCNA)[97], the replication factor Rfc2[98], the histones H2A and H2AX[99] and the Fanconi pathway proteins FA complementation group D2 protein (FANCD2) and FA complementation group I (FANCI)[100]. As anticipated, many UBDs have been found to be involved in the regulation of the DNA repair pathway, by recognizing and processing ubiquitinated proteins (reviewed by Hofmann[101]).

A study by Kim et al. has revealed that the BRCA1, which participates in the DNA damage response, is regulated by receptor-associated protein 80 (RAP80)[102]. Particularly, it was shown that RAP80 relocalizes to damage-induced foci in response to DNA damage and mediates BRCA1 functions after DNA damage. Importantly, the study identified the presence of a tandem UIM domain in RAP80, which mediates interactions with Ub and is required for the localization of RAP80 to sites of DNA breaks, and thus for the regulation of BRCA1[102]. The authors suggest that RAP80, as a protein involved in the DNA damage response pathway, may function as a tumor suppressor protein, and that dysregulation or mutations of RAP80 could trigger human pathophysiology.

Furthermore, several studies have demonstrated that in response to DNA damage, the histones H2A and H2AX are ubiquitinated at the site of damage by two Ub ligases known as RNF8 and RNF168[103–107]. The importance of two UBDs, namely MIU1 and MIU2, for RNF168 localization and thus RNF168 activity, has also been demonstrated[106,107]. Pinato et al. have recently identified a novel UBD, the UMI (UIM-

and MIU-related UBD), present in the Ub ligase RNF168[108]. This study has shown that the UMI domain plays a critical role for the proper localization and function of RNF168 and for the ubiquitination of nuclear proteins, including histone H2A. Several studies have linked abnormalities in histone ubiquitination with the pathogenesis of human diseases, including cancer (reviewed by Cao and Yan[109]). Also, it was demonstrated that inactivation of the UMI domain prevents the recruitment of the downstream mediator 53BP1 at the DNA damage response foci, which is vital for the activation of the downstream effectors of the DNA damage response[108].

UBDs and the nucleotide excision repair pathway

Cockayne syndrome is a rare autosomal-recessive disorder characterized by progressive neurodevelopmental abnormality, premature aging and UV sensitivity[110]. It is associated with defects in the nucleotide excision repair (NER) pathway, particularly in the transcription-coupled NER (TC-NER) subpathway, caused by defects in either of the Cockayne syndrome groups A and B genes (*CSB* and *CSA*, also known as *ERCC6* and *ERCC8*, respectively)[111]. TC-NER specifically removes transcription-blocking DNA damage from the transcribed strands of active genes, thereby promoting the recovery of RNA synthesis after UV irradiation[112]. Although the molecular mechanism that governs the TC-NER function in human cells remains to be clarified, strong evidence supports the notion that CSA and CSB proteins are key regulators of the TC-NER pathway. A recent study by Anindya et al. revealed the presence of a UBD in the C-terminal region of CSB protein, which mediates interactions of CSB with Ub[113]. The study showed that cells expressing CSB protein lacking the UBD have phenotypes similar to those of CSB-deficient cells; this highlights the essential role of Ub binding for CSB function. The authors also suggested that TC-NER requires protein ubiquitination and subsequent recognition by CSB's UBD[113].

UBDs and the FA pathway

FA is a chromosomal instability disorder resulting in the accumulation of DNA damage at an increased rate. The dramatic effects of this disorder upon human health are well demonstrated by the wide range of pathophysiological conditions associated with FA. These include bone marrow failure, congenital abnormalities (including skeletal defects and hypopigmentation) and hematologic malignancies[114]. Interestingly, FA proteins along with the BRCA1 gene product cooperate in the FA-BRCA pathway, which regulates the cellular response to DNA damage and also suppresses cellular transformation pathways[115]. It has also been shown that disruption of the FA-BRCA pathway leads to cellular hypersensitivity to the cytotoxic and clastogenic effects of DNA interstrand crosslinking agents[116]. Crucial to the activation of the FA-BRCA pathway is the covalent mono-ubiquitination of FANCD2 and FANCI proteins, which is mediated by the FA core complex[100,117,118]. Mono-ubiquitinated FANCD2 and FANCI proteins are subsequently driven to distinct chromatin-associated nuclear foci and associate with other key DNA repair proteins, such as BRCA1[117], RAD51[119] and FANCD1/BRCA2[120]. Recent studies have also shown that mono-ubiquitinated FANCD2 protein is involved in the mechanism that mediates the recruitment of FAN1 and SLC4/FANCP endonucleases to sites of DNA damage[121–126]. A recent study has revealed the presence of a coupling of Ub conjugation to endoplasmic reticulum degradation motif (CUE) domain in the N-terminus of FANCD2 protein. The same study has shown that the CUE domain mediates the heterodimerization of FANCD2 with FANCI via non-covalent interaction with the Ub moiety that is covalently attached to K523 residue of FANCI[127]. In addition, the authors suggested that the interaction between FANCD2 and FANCI protects mono-ubiquitinated FANCD2 from polyubiquitination and proteasomal degradation. Importantly, the study highlighted the crucial role of the CUE domain in the regulation of FANCD2 protein, which is involved in bone marrow failure and cancer susceptibility[127].

UBDs and the TLS pathway

Many studies have shown that dysregulation of components of the ubiquitination pathway, including E3 Ub ligases[128–131] and DUBs[132,133], is linked to tumorigenesis. The intrinsic role of UBDs in cancer development has also been revealed. Mammalian cells replicate across DNA lesions via the TLS pathway, which is regulated by either the Ub-binding motif (UBM) or the UBZ domains, which are located at the C-terminus of the Y-family of TLS polymerases (pols)[134]. More specifically, the PCNA is mono-ubiquitinated in response to certain types of DNA damage. Subsequently, recognition of mono-ubiquitinated PCNA by the UBM and UBZ domains of TLS polymerases results in the accumulation of the low-fidelity polymerases pol ι and pol η (error-prone TLS polymerases) at stalled replication forks and eventually in the replacement of the high-fidelity polymerase pol δ; this procedure induces bypass of the DNA lesion[134]. Studies have shown that mutations in either the UBZ or the UBM domain are related to increased UV sensitivity[134,135]. Importantly, it has been shown that patients suffering from Xeroderma pigmentosum variant (XP-V), a syndrome characterized by an increased sensitivity to UV-induced DNA lesions and increased incidence of cancer, are lacking a functional pol η[136,137].

Targeting the ubiquitin pathway

The Ub-proteasome pathway, which degrades the majority of intracellular proteins and thus maintains normal cellular homeostasis, is mediated by the proteasome holoenzyme, Ub ligases and DUB enzymes[1]. Non-lysosomal proteolysis relies on the ability of Ub to label substrates for protein degradation by the proteasome. Previous studies have linked the pathogenesis of various human diseases with deregulation of the Ub-proteasome pathway[128,138]. Inhibition of this regulation mechanism, either at the level of the proteasome, ubiquitination or DUB enzymes, provides new targets for the development of novel therapeutic strategies. Indeed, bortezomib, a boronic acid dipeptide[139], is the first proteasome inhibitor that has been approved for the treatment of multiple myeloma patients. A crystal structure of the yeast 20S proteasome in complex with bortezomib has shown that the latter blocks the

activity of the proteolytically active Thr21 residue; the boron atom of bortezomib is covalently attached to the nucleophilic oxygen lone pair of Thr21[140].

In addition to proteasome inhibitors, more recent studies have focused on the discovery and development of inhibitors of the ubiquitination cycle (via inhibition of E1 and E3 enzymes) and of DUB enzymes, which have recently been linked to several human diseases, including cancer and immune disorders[132,133,141,142]. Moreover, a recent study has reported the inhibition of the DUB enzyme, Ub-specific protease 7 (USP7), by the small molecule P5091 (2-acetyl-4-nitro-5-(2,3-dicloro-phenylsulfanyl)-thiophene)[143]. USP7 stabilizes murine double minute oncogene (MDM2) levels, which consequently mediates p53 ubiquitination and thus its proteasomal degradation. The study has also suggested that P5091 inhibits USP7 activity without blocking proteasome function directly. This inhibition approach, according to the authors, is less likely to trigger off-target activities and associated toxicities.

The Ub-UBD interaction has also been considered as a potential target for the design of novel anticancer drugs. It has been shown that a class of small molecules, known as ubistatins, can prevent the recognition of ubiquitinated proteins by UBDs which are present in the proteasome receptors RPN10 and RPN13[144,145]. Particularly, ubistatins target the hydrophobic patch of Lys48-linked chains, which is used to regulate interactions of Lys48-linked chains with multiple UBD-containing proteins[144]. Recently, it has been reported that the NF-κB pathway, which is involved in innate and adaptive immunity, oncogenesis and development[146–149], can be selectively inhibited by a peptide inhibitor, termed Ub-binding inhibitor (UBI)[150]. The study showed that UBI disrupts the interaction between NEMO Optineurin ABIN (NOA) UBD of NEMO and Lys63-linked polyUb chains, resulting in the inhibition of NF-κB activation.

Conclusions

Ubiquitination, a fundamental PTM resulting in the covalent attachment of Ub to a target protein, is implicated in several key cellular processes. Substantial evidence currently shows that proteins labeled with polyUb chains of specific topology and length are driven to a specific cellular process. Therefore, the diversity of ubiquitination can be attributed to the ability of Ub to utilize any one of its seven Lys residues or its N-terminus to form polyUb chains of specific Lys linkage. The sorting and processing of distinct Ub signals is carried out by small protein motifs, known as UBDs, which are found in proteins that execute disparate biological functions. Functional impairment of UBDs in key regulatory pathways is associated with human pathophysiology and development of disease. Interestingly, the involvement of UBDs in the pathogenesis of human disorders makes the Ub-UBD interaction a potential target for the development of novel therapeutic agents. In this review, we presented an updated account of the escalating importance of UBDs and their functions, with a special emphasis on their roles in the pathophysiology of several disorders. Finally, current studies targeting the inhibition of specific Ub-UBD interactions were discussed.

Declaration of interest

KS is financially supported by the Project NEW INFRASTRUCTURE/NEKYP/0311/17, which is co-financed by the European Regional Development Fund and the Republic of Cyprus through the Research Promotion Foundation.

References

1. Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 1998;67:425–79.
2. Varshavsky A. Regulated protein degradation. *Trends Biochem Sci* 2005;30:283–6.
3. Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* 2007;315:201–5.
4. Hirsch C, Gauss R, Horn SC, et al. The ubiquitylation machinery of the endoplasmic reticulum. *Nature* 2009;458:453–60.
5. Vucic D, Dixit VM, Wertz IE. Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death. *Nat Rev Mol Cell Biol* 2011;12:439–52.
6. Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. *Mol Cell* 2009;34:259–69.
7. Elsasser S, Finley D. Delivery of ubiquitinated substrates to protein-unfolding machines. *Nat Cell Biol* 2005;7:742–9.
8. Staub O, Rotin D. Role of ubiquitylation in cellular membrane transport. *Physiol Rev* 2006;86:669–707.
9. Huang TT, D'Andrea AD. Regulation of DNA repair by ubiquitylation. *Nat Rev Mol Cell Biol* 2006;7:323–34.
10. Raiborg C, Bache KG, Gillooly DJ, et al. Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nat Cell Biol* 2002;4:394–8.
11. Hurley JH, Lee S, Prag G. Ubiquitin-binding domains. *Biochem J* 2006;399:361–72.
12. Hicke L, Schubert HL, Hill CP. Ubiquitin-binding domains. *Nat Rev Mol Cell Biol* 2005;6:610–21.
13. Dikic I, Wakatsuki S, Walters KJ. Ubiquitin-binding domains – from structures to functions. *Nat Rev Mol Cell Biol* 2009;10:659–71.
14. Komander D, Rape M. The ubiquitin code. *Annu Rev Biochem* 2012;81:203–29.
15. Ciechanover A, Ben-Saadon R. N-terminal ubiquitination: more protein substrates join in. *Trends Cell Biol* 2004;14:103–6.
16. Ravid T, Hochstrasser M. Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue. *Nat Cell Biol* 2007;9:422–7.
17. Wang XL, Herr RA, Chua WJ, et al. Ubiquitination of serine, threonine, or lysine residues on the cytoplasmic tail can induce ERAD of MHC-I by viral E3 ligase mK3. *J Cell Biol* 2007;177:613–24.
18. Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001;70:503–33.
19. Chau V, Tobias JW, Bachmair A, et al. A multiubiquitin chain is confined to specific lysise in a targeted short-lived protein. *Science* 1989;243:1576–83.
20. Cook WJ, Jeffrey LC, Carson M, et al. Structure of a diubiquitin conjugate and a model for interaction with ubiquitin conjugating enzyme (E2). *J Biol Chem* 1992;267:16467–71.
21. Sun LJ, Chen ZJ. The novel functions of ubiquitination in signaling. *Curr Opin Cell Biol* 2004;16:119–26.
22. Hicke L, Dunn R. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu Rev Cell Dev Biol* 2003;19:141–72.
23. Spence J, Gali RR, Dittmar G, et al. Cell cycle-regulated modification of the ribosome by a variant multiubiquitin chain. *Cell* 2000;102:67–76.
24. Komander D. The emerging complexity of protein ubiquitination. *Biochem Soc Trans* 2009;37:937–53.
25. Wu-Baer F, Lagrazon K, Yuan W, Baer R. The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. *J Biol Chem* 2003;278:34743–6.

26. Morris JR, Solomon E. BRCA1: BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum Mol Genet* 2004;13: 807–17.
27. Kirisako T, Kamei K, Murata S, et al. A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J* 2006; 25:4877–87.
28. Iwai K, Tokunaga F. Linear polyubiquitination: a new regulator of NF-kappaB activation. *EMBO Rep* 2009;10:706–13.
29. Tokunaga F, Sakata S, Saeki Y, et al. Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* 2009;11:123–32.
30. Rahighi S, Ikeda F, Kawasaki M, et al. Specific recognition of linear ubiquitin chains by NEMO is important for NF-κB activation. *Cell* 2009;136:1098–109.
31. Nakasone Mark A, Livnat-Levanon N, Glickman Michael H, et al. Mixed-linkage ubiquitin chains send mixed messages. *Structure (London, England: 1993)* 2013;21:727–40.
32. Ikeda F, Dikic I. Atypical ubiquitin chains: new molecular signals – 'protein modifications: beyond the usual suspects' review series. *EMBO Rep* 2008;9:536–42.
33. Husnjak K, Dikic I. Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. *Annu Rev Biochem* 2012; 81:291–322.
34. Kulathu Y, Komander D. Atypical ubiquitylation – the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat Rev Mol Cell Biol* 2012;13:508–23.
35. Massey LK, Mah AL, Ford DL, et al. Overexpression of ubiquilin decreases ubiquitination and degradation of presenilin proteins. *J Alzheimers Dis* 2004;6:79–92.
36. Raasi S, Pickart CM. Rad23 ubiquitin-associated domains (UBA) inhibit 26 S proteasome-catalyzed proteolysis by sequestering lysine 48-linked polyubiquitin chains. *J Biol Chem* 2003;278: 8951–9.
37. Brignone C, Bradley KE, Kisselev AF, Grossman SR. A post-ubiquitination role for MDM2 and hHR23A in the p53 degradation pathway. *Oncogene* 2004;23:4121–9.
38. Walters KJ, Lech PJ, Goh AM, et al. DNA-repair protein hHR23a alters its protein structure upon binding proteasomal subunit S5a. *Proc Natl Acad Sci USA* 2003;100:12694–9.
39. Deveraux Q, Ustrell V, Pickart C, Rechsteiner M. A 26-S protease subunit that binds ubiquitin conjugates. *J Biol Chem* 1994;269: 7059–61.
40. Fisher RD, Wang B, Alam SL, et al. Structure and ubiquitin binding of the ubiquitin-interacting motif. *J Biol Chem* 2003;278: 28976–84.
41. Lee S, Tsai YC, Mattera R, et al. Structural basis for ubiquitin recognition and autoubiquitination by Rabex-5. *Nat Struct Mol Biol* 2006;13:264–71.
42. Hirano S, Kawasaki M, Ura H, et al. Double-sided ubiquitin binding of Hrs-UIM in endosomal protein sorting. *Nat Struct Mol Biol* 2006;13:272–7.
43. Haririnia A, Verma R, Purohit N, et al. Mutations in the hydrophobic core of ubiquitin differentially affect its recognition by receptor proteins. *J Mol Biol* 2008;375:979–96.
44. Penengo L, Mapelli M, Murachelli AG, et al. Crystal structure of the ubiquitin binding domains of rabex-5 reveals two modes of interaction with ubiquitin. *Cell* 2006;124:1183–95.
45. VanDemark AP, Hofmann RM, Tsui C, et al. Molecular insights into polyubiquitin chain assembly: crystal structure of the Mms2/Ubc13 heterodimer. *Cell* 2001;105:711–20.
46. Wu T, Merbl Y, Huo Y, et al. UBE2S drives elongation of K11-linked ubiquitin chains by the anaphase-promoting complex. *Proc Natl Acad Sci USA* 2010;107:1355–60.
47. Chen Z, Pickart CM. A 25-kilodalton ubiquitin carrier protein (E2) catalyzes multi-ubiquitin chain synthesis via lysine 48 of ubiquitin. *J Biol Chem* 1990;265:21835–42.
48. Wilson RC, Edmondson SP, Flatt JW, et al. The E2-25K ubiquitin-associated (UBA) domain aids in polyubiquitin chain synthesis and linkage specificity. *Biochem Biophys Res Commun* 2011;405: 662–6.
49. Song S, Kim S-Y, Hong Y-M, et al. Essential role of E2-25K/Hip-2 in mediating amyloid-β neurotoxicity. *Mol Cell* 2003;12: 553–63.
50. Barnham KJ, Cappai R, Beyreuther K, et al. Delineating common molecular mechanisms in Alzheimer's and prion diseases. *Trends Biochem Sci* 2006;31:465–72.
51. van Leeuwen FW, de Kleijn DP, van den Hurk HH, et al. Frameshift mutants of beta amyloid precursor protein and ubiquitin-B in Alzheimer's and Down patients. *Science* 1998; 279:242–7.
52. De Vrij FM, Sluijs JA, Gregori L, et al. Mutant ubiquitin expressed in Alzheimer's disease causes neuronal death. *FASEB J* 2001;15: 2680–8.
53. Lindsten K, de Vrij FMS, Verhoef LGGC, et al. Mutant ubiquitin found in neurodegenerative disorders is a ubiquitin fusion degradation substrate that blocks proteasomal degradation. *J Cell Biol* 2002;157:417–27.
54. Ko S, Kang GB, Song SM, et al. Structural basis of E2-25K/UBB + 1 interaction leading to proteasome inhibition and neurotoxicity. *J Biol Chem* 2010;285:36070–80.
55. de Pril R, Fischer DF, Roos RA, van Leeuwen FW. Ubiquitin-conjugating enzyme E2-25K increases aggregate formation and cell death in polyglutamine diseases. *Mol Cell Neurosci* 2007;34: 10–19.
56. Reyes-Turcu FE, Horton JR, Mullally JE, et al. The ubiquitin binding domain ZnFUBP recognizes the C-terminal diglycine motif of unanchored ubiquitin. *Cell* 2006;124:1197–208.
57. Sokratous K, Roach LV, Channing D, et al. Probing affinity and ubiquitin linkage selectivity of ubiquitin-binding domains using mass spectrometry. *J Am Chem Soc* 2012; 134:6416–24.
58. Raasi S, Orlov I, Fleming KG, Pickart CM. Binding of polyubiquitin chains to ubiquitin-associated (UBA) domains of HHR23A. *J Mol Biol* 2004;341:1367–79.
59. Raasi S, Varadan R, Fushman D, Pickart CM. Diverse polyubiquitin interaction properties of ubiquitin-associated domains. *Nat Struct Mol Biol* 2005;12:708–14.
60. Varadan R, Assfalg M, Raasi S, et al. Structural determinants for selective recognition of a lys48-linked polyubiquitin chain by a UBA domain. *Mol Cell* 2005;18:687–98.
61. Castaneda CA, Kashyap TR, Nakasone MA, et al. Unique structural, dynamical, and functional properties of k11-linked polyubiquitin chains. *Structure* 2013;21:1168–81.
62. Zhang D, Raasi S, Fushman D. Affinity makes the difference: nonselective interaction of the UBA domain of ubiquilin-1 with monomeric ubiquitin and polyubiquitin chains. *J Mol Biol* 2008; 377:162–80.
63. Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. *Sci STKE* 2006;2006:re13.
64. Wu CJ, Conze DB, Li T, et al. Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation [corrected]. *Nat Cell Biol* 2006;8:398–406.
65. Ea CK, Deng L, Xia ZP, et al. Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. *Mol Cell* 2006;22:245–57.
66. Bloor S, Ryzhakov G, Wagner S, et al. Signal processing by its coil zipper domain activates IKK gamma. *Proc Natl Acad Sci USA* 2008;105:1279–84.
67. Wagner S, Carpentier I, Rogov V, et al. Ubiquitin binding mediates the NF-kappaB inhibitory potential of ABIN proteins. *Oncogene* 2008;27:3739–45.
68. Lo YC, Lin SC, Rospigliosi CC, et al. Structural basis for recognition of diubiquitins by NEMO. *Mol Cell* 2009;33:602–15.
69. Doffinger R, Smahi A, Bessia C, et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nat Genet* 2001;27:277–85.
70. Filipe-Santos O, Bustamante J, Haverkamp MH, et al. X-linked susceptibility to mycobacteria is caused by mutations in NEMO impairing CD40-dependent IL-12 production. *J Exp Med* 2006; 203:1745–59.
71. Song HY, Rothe M, Goeddel DV. The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-kappaB activation. *Proc Natl Acad Sci USA* 1996; 93:6721–5.
72. Wang YY, Li L, Han KJ, et al. A20 is a potent inhibitor of TLR3- and Sendai virus-induced activation of NF-kappaB and ISRE and IFN-beta promoter. *FEBS Lett* 2004;576:86–90.

73. Sarma V, Lin Z, Clark L, et al. Activation of the B-cell surface receptor CD40 induces A20, a novel zinc finger protein that inhibits apoptosis. *J Biol Chem* 1995;270:12343–6.
74. Fries KL, Miller WE, Raab-Traub N. The A20 protein interacts with the Epstein–Barr virus latent membrane protein 1 (LMP1) and alters the LMP1/TRAF1/TRADD complex. *Virology* 1999; 264:159–66.
75. Graham RR, Cotsapas C, Davies L, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet* 2008;40:1059–61.
76. Musone SL, Taylor KE, Lu TT, et al. Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. *Nat Genet* 2008;40:1062–4.
77. Hammer GE, Turer EE, Taylor KE, et al. Expression of A20 by dendritic cells preserves immune homeostasis and prevents colitis and spondyloarthritis. *Nat Immunol* 2011;12:1184–93.
78. Vereecke L, Sze M, Mc Guire C, et al. Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor-induced toxicity and experimental colitis. *J Exp Med* 2010;207:1513–23.
79. Matmati M, Jacques P, Maelfait J, et al. A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat Genet* 2011;43:908–12.
80. Kool M, van Loo G, Waelput W, et al. The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells, and systemic autoimmunity. *Immunity* 2011;35: 82–96.
81. Tavares RM, Turer EE, Liu CL, et al. The ubiquitin modifying enzyme A20 restricts B cell survival and prevents autoimmunity. *Immunity* 2010;33:181–91.
82. Shembade N, Ma A, Harhaj EW. Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. *Science* 2010;327:1135–9.
83. Wertz IE, O'Rourke KM, Zhou H, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* 2004;430:694–9.
84. Skaug B, Chen J, Du F, et al. Direct, noncatalytic mechanism of IKK inhibition by A20. *Mol Cell* 2011;44:559–71.
85. Verhelst K, Carpentier I, Kreike M, et al. A20 inhibits LUBAC-mediated NF-κB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J* 2012;31:3845–55.
86. Kato M, Sanada M, Kato I, et al. Frequent inactivation of A20 in B-cell lymphomas. *Nature* 2009;459:712–16.
87. Schmitz R, Hansmann ML, Bohle V, et al. TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma. *J Exp Med* 2009;206:981–9.
88. Hunter AM, LaCasse EC, Korneluk RG. The inhibitors of apoptosis (IAPs) as cancer targets. *Apoptosis* 2007;12:1543–68.
89. Gyrd-Hansen M, Darding M, Miasari M, et al. IAPs contain an evolutionarily conserved ubiquitin-binding domain that regulates NF-kappaB as well as cell survival and oncogenesis. *Nat Cell Biol* 2008;10:1309–17.
90. Layfield R, Ciani B, Ralston SH, et al. Structural and functional studies of mutations affecting the UBA domain of SQSTM1 (p62) which cause Paget's disease of bone. *Biochem Soc Trans* 2004;32: 728–30.
91. Cavey JR, Ralston SH, Sheppard PW, et al. Loss of ubiquitin binding is a unifying mechanism by which mutations of SQSTM1 cause Paget's disease of bone. *Calcif Tissue Int* 2006;78:271–7.
92. Visconti MR, Langston AL, Alonso N, et al. Mutations of SQSTM1 are associated with severity and clinical outcome in paget disease of bone. *J Bone Miner Res* 2010;25:2368–73.
93. Michou L, Collet C, Laplanche JL, et al. Genetics of Paget's disease of bone. *Joint Bone Spine* 2006;73:243–8.
94. Garner TP, Long J, Layfield R, Searle MS. Impact of p62/ SQSTM1 UBA domain mutations linked to Paget's disease of bone on ubiquitin recognition. *Biochemistry* 2011;50:4665–74.
95. Goode A, Layfield R. Recent advances in understanding the molecular basis of Paget disease of bone. *J Clin Pathol* 2010;63: 199–203.
96. Rea SL, Walsh JP, Layfield R, et al. New insights into the role of sequestosome 1/p62 mutant proteins in the pathogenesis of Paget's disease of bone. *Endocr Rev* 2013;34:501–24.
97. Hoege C, Pfander B, Moldovan GL, et al. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 2002;419:135–41.
98. Tomida J, Masuda Y, Hiroaki H, et al. DNA damage-induced ubiquitylation of RFC2 subunit of replication factor C complex. *J Biol Chem* 2008;283:9071–9.
99. Bergink S, Salomons FA, Hoogstraten D, et al. DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A. *Genes Dev* 2006;20:1343–52.
100. Smogorzewska A, Matsuoka S, Vinciguerra P, et al. Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell* 2007;129:289–301.
101. Hofmann K. Ubiquitin-binding domains and their role in the DNA damage response. *DNA Repair (Amst)* 2009;8:544–56.
102. Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science* 2007;316: 1202–5.
103. Kolas NK, Chapman JR, Nakada S, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 2007;318:1637–40.
104. Mailand N, Bekker-Jensen S, Faustrup H, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 2007;131:887–900.
105. Huen MS, Grant R, Manke I, et al. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 2007;131:901–14.
106. Doil C, Mailand N, Bekker-Jensen S, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* 2009;136: 435–46.
107. Pinato S, Scandiuzzi C, Arnaudo N, et al. RNF168, a new RING finger, MIU-containing protein that modifies chromatin by ubiquitination of histones H2A and H2AX. *BMC Mol Biol* 2009;10:55.
108. Pinato S, Gatti M, Scandiuzzi C, et al. UMI, a novel RNF168 ubiquitin binding domain involved in the DNA damage signaling pathway. *Mol Cell Biol* 2011;31:118–26.
109. Cao J, Yan Q. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. *Front Oncol* 2012;2:26.
110. Lehmann AR. DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. *Biochimie* 2003;85:1101–11.
111. Licht CL, Stevnsner T, Bohr VA. Cockayne syndrome group B cellular and biochemical functions. *Am J Hum Genet* 2003;73: 1217–39.
112. Hanawalt PC, Spivak G. Transcription-coupled DNA repair: two decades of progress and surprises. *Nat Rev Mol Cell Biol* 2008;9: 958–70.
113. Anindya R, Mari P-O, Kristensen U, et al. A ubiquitin-binding domain in Cockayne syndrome B required for transcription-coupled nucleotide excision repair. *Mol Cell* 2010;38:637–48.
114. Taniguchi T, D'Andrea AD. Molecular pathogenesis of Fanconi anemia: recent progress. *Blood* 2006;107:4223–33.
115. Moldovan G-L, D'Andrea AD. How the Fanconi anemia pathway guards the genome. *Annu Rev Genet* 2009;43:223–49.
116. Auerbach AD, Wolman SR. Susceptibility of Fanconi's anaemia fibroblasts to chromosome damage by carcinogens. *Nature* 1976; 261:494–6.
117. Garcia-Higuera I, Taniguchi T, Ganesan S, et al. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol Cell* 2001;7:249–62.
118. Sims AE, Spiteri E, Sims RJ, et al. FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. *Nat Struct Mol Biol* 2007;14:564–7.
119. Taniguchi T, Garcia-Higuera I, Andreassen PR, et al. S-phase-specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51. *Blood* 2002;100:2414–20.
120. Wang X, Andreassen PR, D'Andrea AD. Functional interaction of monoubiquitinated FANCD2 and BRCA2/FANCD1 in chromatin. *Mol Cell Biol* 2004;24:5850–62.
121. Cybulski KE, Howlett NG. FANCP/SLX4: a Swiss army knife of DNA interstrand crosslink repair. *Cell Cycle* 2011;10:1757–63.
122. Kim Y, Lach FP, Desetty R, et al. Mutations of the SLX4 gene in Fanconi anemia. *Nat Genet* 2011;43:142–6.
123. Kratz K, Schopf B, Kaden S, et al. Deficiency of FANCD2-associated nuclease KIAA1018/FAN1 sensitizes cells to interstrand crosslinking agents. *Cell* 2010;142:77–88.

124. MacKay C, Declais AC, Lundin C, et al. Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. *Cell* 2010;142:65–76.
125. Smogorzewska A, Desetty R, Saito TT, et al. A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. *Mol Cell* 2010;39:36–47.
126. Stoepker C, Hain K, Schuster B, et al. SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype. *Nat Genet* 2011;43:138–41.
127. Rego MA, Kolling FW, Vuono EA, et al. Regulation of the Fanconi anemia pathway by a CUE ubiquitin-binding domain in the FANCD2 protein. *Blood* 2012;120:2109–17.
128. Hoeller D, Hecker CM, Dikic I. Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat Rev Cancer* 2006;6:776–88.
129. Nakayama KI, Nakayama K. Ubiquitin ligases: cell-cycle control and cancer. *Nat Rev Cancer* 2006;6:369–81.
130. Bernassola F, Karin M, Ciechanover A, Melino G. The HECT family of E3 ubiquitin ligases: multiple players in cancer development. *Cancer Cell* 2008;14:10–21.
131. Satija YK, Bhardwaj A, Das S. A portrayal of E3 ubiquitin ligases and deubiquitylases in cancer. *Int J Cancer* 2013;133: 2759–68.
132. Sgorbissa A, Potu H, Brancolini C. Isopeptidases in anticancer therapy: looking for inhibitors. *Am J Transl Res* 2010;2:235–47.
133. Nicholson B, Marblestone JG, Butt TR, Mattern MR. Deubiquitinating enzymes as novel anticancer targets. *Future Oncol* 2007;3:191–9.
134. Bienko M, Green CM, Crosetto N, et al. Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science* 2005;310:1821–4.
135. Guo C, Tang TS, Bienko M, et al. Ubiquitin-binding motifs in REV1 protein are required for its role in the tolerance of DNA damage. *Mol Cell Biol* 2006;26:8892–900.
136. Masutani C, Kusumoto R, Yamada A, et al. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature* 1999;399:700–4.
137. Johnson RE, Kondratick CM, Prakash S, Prakash L. hRAD30 mutations in the variant form of xeroderma pigmentosum. *Science* 1999;285:263–5.
138. Adams J. The proteasome: a suitable antineoplastic target. *Nat Rev Cancer* 2004;4:349–60.
139. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* 2003; 348:2609–17.
140. Groll M, Berkers CR, Ploegh HL, Ovaa H. Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20S proteasome. *Structure* 2006;14:451–6.
141. Hoeller D, Dikic I. Targeting the ubiquitin system in cancer therapy. *Nature* 2009;458:438–44.
142. Fulda S, Rajalingam K, Dikic I. Ubiquitylation in immune disorders and cancer: from molecular mechanisms to therapeutic implications. *EMBO Mol Med* 2012;4:545–56.
143. Chauhan D, Tian Z, Nicholson B, et al. A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes Bortezomib resistance. *Cancer Cell* 2012;22:345–58.
144. Verma R, Peters NR, D'Onofrio M, et al. Ubistatins inhibit proteasome-dependent degradation by binding the ubiquitin chain. *Science* 2004;306:117–20.
145. Husnjak K, Elsasser S, Zhang NX, et al. Proteasome subunit Rpn13 is a novel ubiquitin receptor. *Nature* 2008;453:481–8.
146. Hayden MS, Ghosh S. Shared principles in NF-B signaling. *Cell* 2008;132:344–62.
147. Perkins ND. Integrating cell-signalling pathways with NF-kappa B and IKK function. *Nat Rev Mol Cell Biol* 2007;8:49–62.
148. Scheidereit C. I kappa B kinase complexes: gateways to NF-kappa B activation and transcription. *Oncogene* 2006;25:6685–705.
149. Vallabhapurapu S, Karin M. Regulation and function of NF-κB transcription factors in the immune system. *Annu Rev Immunol* 2009;27:693–733.
150. Chiaravalli J, Fontan E, Fsihi H, et al. Direct inhibition of NF-κB activation by peptide targeting the NOA ubiquitin binding domain of NEMO. *Biochem Pharmacol* 2011;82:1163–74.
151. Cornilescu G, Marquardt JL, Ottiger M, Bax A. Validation of protein structure from anisotropic carbonyl chemical shifts in a dilute liquid crystalline phase. *J Am Chem Soc* 1998;120:6836–7.
152. Alam SL, Sun J, Payne M, et al. Ubiquitin interactions of NZF zinc fingers. *EMBO J* 2004;23:1411–21.