Linear ubiquitin chain-binding domains

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Ubiquitin modification (ubiquitination) of target proteins can vary with respect to chain lengths, linkage type, and chain forms, such as homologous, mixed, and branched ubiquitin chains. Thus, ubiquitination can generate multiple unique surfaces on a target protein substrate. Ubiquitin-binding domains (UBDs) recognize ubiquitinated substrates, by specifically binding to these unique surfaces, modulate the formation of cellular signaling complexes and regulate downstream signaling cascades. Among the eight different homotypic chain types, Met1-linked (also termed linear) chains are the only chains in which linkage occurs on a non-Lys residue of ubiquitin. Linear ubiquitin chains have been implicated in immune responses, cell death and autophagy, and several UBDs - specific for linear ubiquitin chains - have been identified. In this review, we describe the main principles of ubiquitin recognition by UBDs, focusing on linear ubiquitin chains and their roles in biology.

Introduction

Ubiquitin, an 8.5 kDa protein conserved from yeast to human, is utilized to post-translationally modify substrate proteins [1,2]. Covalent attachment of ubiquitin to a protein (ubiquitination) regulates their activity, stability, and folding, and thus biological functions, in different species [3]. Unlike other post-translational modifications, such as acetylation or phosphorylation, ubiquitination adds a complex protein tag to its substrate. To date, more than 10 000 ubiquitination sites in over 4000 human proteins have been uncovered by mass spectrometry analysis [4].

Like other post-translational modifications such as phosphorylation and methylation, ubiquitination is reversible (Fig. 1A). Substrate ubiquitination is achieved by a multistep ATP-dependent enzymatic cascade catalyzed by an E1 activating enzyme, an E2 conjugating enzyme and an E3 ligase [5], whereas deubiquitination is performed in a single step by deubiquitinases (DUBs) (Fig. 1A). In humans, two genes encode E1s, ~25 genes encode E2s, and >600 genes encode E3s. The nature of the ubiquitin modification depends on the E2 enzyme and the E3 ligase. Ubiquitin itself can also be post-translationally modified by phosphorylation, acetylation, sumoylation, and neddylation (Fig. 1B) [6]. The roles of post-translationally modified ubiquitin are not well-understood. However, it is clear that phosphorylation of ubiquitin by PTEN-induced “Putative Kinase 1” (PINK1) plays a critical role in regulating mitophagy, a mitochondrial damage response [7,8].

Abbreviations
ABIN, A20-binding inhibitors of NF-kappaB; CYLD, cylindromatosis; DUB, deubiquitinating enzyme; HOIL-1L, haeme-oxidized IRP2 ubiquitin ligase 1L; LUBAC, linear ubiquitin chain assembly complex; NEMO, NF-kappaB essential modulator; OPTINEURIN, optic neuropathy-inducing protein; OTU, ovarian tumor; OTULIN, OTU deubiquitinase with linear linkage specificity; RIPK1, receptor interacting protein kinase 1; TNF, tumor necrosis factor; UBAN, UBD in ABIN proteins and NEMO; UBD, ubiquitin-binding domain; USP, ubiquitin specific protease; ZF, zinc finger.
A single ubiquitin moiety can be conjugated on a substrate (monoubiquitination) (Fig. 1C). Modification of ubiquitin by phosphorylation, acetylation, sumoylation, or neddylation can occur before or after the monoubiquitination event is completed (Fig. 1C). Ubiquitin chains are established via conjugation to intrinsic ubiquitin residues, including seven Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, or Lys63) or the N-terminal Met1 residue [6,9,10]. This process yields eight possible linkage types within homologous, mixed, or branched chains (Fig. 1D,E) [11]. Ubiquitin chains may contain a mixture of modified and nonmodified ubiquitins (Fig. 1F,G). This variation is important for mediating spatiotemporal regulation of cellular signaling. Ubiquitination not only alters the substrate protein’s binding partners, but may also directly induce conformational changes within the substrate protein [12,13], influencing critical steps of cellular signaling pathways.

Ubiquitin-binding domains (UBDs)

A wide range of proteins with “Ubiquitin-Binding Domains” (UBDs) recognize diverse ubiquitination types on specific substrate sites, thereby forming distinct signaling complexes (Fig. 2A–D) [14]. Thus far, over 20 different families of UBDs have been identified [15,16]. UBD-containing proteins interact with ubiquitin monomers or chains on substrates. Ubiquitin-UBD interactions may occur in trans (between two molecules) (Fig. 2A) or in cis (within a molecule) (Fig. 2B). Recruitment of UBD-containing proteins into a signaling complex via ubiquitin-binding regulates downstream signaling pathways [15]. Ubiquitination of a protein with an intrinsic UBD domain may influence its folding [17], which in turn affects its activity [13] or its interacting partners [16,18,19] to impact signaling cascades.

As mentioned above, recent studies have shown that ubiquitin can be phosphorylated, acetylated, sumoylated, and neddylated. These modifications of ubiquitin potentially alter the UBD-binding surface or chain formation [20–22], therefore it is tempting to speculate that there are distinct UBDs recognizing chains with modified ubiquitins as a monomer or as a chain (Fig. 2C,D). The identification of such UBD-containing proteins would extend our knowledge of ubiquitin biology and its roles in regulating physiology and disease. Alternatively, the post-translational modification of ubiquitin might inhibit canonical ubiquitin-UBD interactions.

UBDs that bind to ubiquitin chains are often selective for a specific type of chain linkage. This selectivity may arise from the recognition of a unique orientation of the chain and distinct surfaces on the ubiquitin moieties, or via direct interaction with the linker region connecting the two ubiquitins. Based on the structural analysis of UBDs, required length of ubiquitin chains to confer specificity to a UBD is suggested to be rather short, either dimeric or trimeric [9,19,23–25]. UBDs in ubiquitin-binding proteins generally occur as single domains or in tandem (Fig. 2A). Tandem “Ubiquitin-Interacting Motifs” (UIMs) in Rap80 and “Arsenite-Inducible RNA-Associated Protein-Like” (AIRAPL) proteins bind cooperatively to Lys63- and Lys48-linked ubiquitin chains respectively [26,27]. An individual UBD may also bind multiple ubiquitin molecules, such as the single “Motif Interacting with Ubiquitin” (MIU) domain within the “MIU-containing Novel DUB family member” (MINDY), which binds to three ubiquitin molecules linked through Lys48 [28]. Also, a double-sided UIM in the “Hepatocyte growth factor-regulated tyrosine kinase substrate” (Hrs) protein is known to bind two independent ubiquitin molecules on either side of an α-helix [29].

Linear ubiquitin chain-specific binding domains and their biological functions

Linear ubiquitin chains linked via Met1 are the only non-Lys residue-dependent type of chain linkage, and thus are biochemically and structurally unique. Linear ubiquitination plays important roles in regulating immunity and cell death signaling cascades, as well as autophagy (Fig. 3A–B) [30–37]. Thus far, the only E3 ligase complex known to generate linear ubiquitin chains is the “Linear Ubiquitin Chain Assembly Complex” (LUBAC), which consists of “Haeme-Oxidized IRP2 ubiquitin Ligase 1L” (HOIL-1L), “HOIL-1-Interacting Protein” (HOIP), and “Shank-Associated RH Domain-Interacting Protein” (SHARPIN) (Fig. 3A,B) [38–40]. HOIP is a “RING-in-Between-RING” (RBR)-type E3 ligase, and its C-terminal “Linear ubiquitin chain Determining Domain” (LDD) is critical for the formation of linear/Met1-linked ubiquitin chains [41]. Like many other RBR-type E3 ligases, HOIP is autoinhibited, and becomes catalytically active upon binding to HOIL-1L or SHARPIN, presumably due to conformational changes in HOIP [42]. Interestingly, the HOIP-RBR domain does not require HOIL-1L or SHARPIN to catalyze at least unanchored linear ubiquitin chains in vitro [42]. Linear ubiquitin chains can be hydrolyzed by the DUBs “OTU DUB with linear linkage specificity” (OTULIN) [43,44] and Cylindromatosis (CYLD) (Fig. 3A,B) [45,46].
Ubiquitin chains with linear linkages establish distinct interactions with specific UBDs. Thus far, seven proteins harboring linear ubiquitin chain-specific UBDs are known, including HOIL-1L, OTULIN, and CYLD (Fig. 4A–G). All of these proteins have been implicated in regulating the signaling...
pathways that involve linear ubiquitination (Fig. 3A–B) [31–34].

The “UBD in ABIN proteins and NEMO” (UBAN) domain

**NEMO-UBAN**

The UBAN domain in “NF-κB essential modulator” (NEMO)/”IκB kinase gamma” (IKKγ) was the first linear ubiquitin chain-specific binding domain identified [47] (Fig. 4A), and has been structurally analyzed by x-ray crystallography (Fig. 5A) [19,48]. The C-terminal zinc finger (ZF) of NEMO co-immunoprecipitates with polyubiquitinated signaling components, and thus NEMO was speculated to interact with Lys63-linked ubiquitin chains. Unexpectedly, NEMO-UBAN interacts with linear diubiquitin chains with approximately 100-fold higher affinity compared to Lys63-linked diubiquitin chains [19,49]. NEMO-UBAN forms a parallel homodimer that adopts a highly symmetric coiled-coil structure, resulting in the simultaneous binding of two linear diubiquitin chains along NEMO-UBAN (Fig. 5A). NEMO binds to the distal and proximal ubiquitins within a linear diubiquitin via distinct surfaces centered on Ile44 and Phe4.
respectively (Fig. 5A). Furthermore, tight interactions between NEMO-UBAN and the C-terminal tail of the distal ubiquitin (residues Leu71-Arg74) that forms a linker between two ubiquitin molecules ensures its specificity for linear ubiquitin chains. NEMO plays an important role as part of the “IκB Kinase” (IKK) complex in the “Nuclear Factor-KappaB” (NF-κB) activation signaling cascade, both as a linear ubiquitination substrate and linear ubiquitin chain binding protein (Fig. 3A). The interaction of linear ubiquitin chains with NEMO-UBAN alters the conformation of NEMO [17,19], which may have an allosteric effect, regulating IKK kinase activity and downstream signaling. Genetic [19] and pharmacological [50] inhibition
of the interaction between NEMO-UBAN and linear ubiquitin chains preclude the full activation of NF-κB, revealing an important role for the NEMO-linear ubiquitin chain interaction in regulating the NF-κB pathway. Whether NEMO-UBAN binds to linear ubiquitination formed on NEMO itself, and whether this interaction affects the oligomeric state of the IKK complex, remain unclear.

Patients suffering from incontinentia pigmenti and “Ectodermal Dysplasia with Immuno Deficiency” (EDA-ID) display mutations in the NEMO gene, including at positions within the UBAN domain (Fig. 4A, Fig. 5A, Table 1) [51–53]. Interestingly, some of these mutations (Asp311Asn, Glu315Ala, and Arg319Gln, corresponding to Asp304Asn, Glu308Ala and Arg312Gln in mouse NEMO as in Fig. 5A) prevent the interaction between NEMO-UBAN and linear ubiquitin chains, suggesting that loss of this interaction might underlie this disease [19].

**ABIN-1, 2, 3-UBAN**

The three “A20-Binding Inhibitors of NF-κB” (ABIN) proteins are known to inhibit the NF-κB pathway and the ABIN-UBAN domains are important for this regulation (Fig. 5A and 4B) [54]. The ABIN proteins are implicated in immunity and cell death based on the analysis of genetically modified mouse models and cellular assays [47]. The three ABIN proteins appear to play nonredundant roles in NF-κB signaling. For example, NF-κB activation induces the expression of ABIN-1 and ABIN-3 but not ABIN-2 [47], suggesting distinct functions in cell signaling regulation.

The UBAN domains in the ABIN proteins were first identified in 2008 [54] and shown to negatively regulate NF-κB signaling. However, a structural understanding of the ABIN-UBAN domain remained unclear until recently. A crystal structure of the ABIN-2-UBAN domain in complex with a linear triubiquitin chain indicated that this domain forms a coiled-coiled homodimer that provides binding sites for two of the ubiquitins, similar to NEMO-UBAN (Fig. 5B,C) [24]. From each triubiquitin chain, the distal ubiquitin binds the UBAN domain through its Ile44 surface. The middle ubiquitin employs both the Phe4- and Ile-44 centered surfaces, acting as the proximal ubiquitin for the same UBAN domain and the distal ubiquitin for the second UBAN dimer from the neighboring asymmetric unit. The third ubiquitin in the chain forms the proximal ubiquitin for binding to the second UBAN dimer (Fig. 5B). Although each ABIN2-UBAN dimer appears to bind one linear triubiquitin in solution as well as in each asymmetric unit of the crystal, the crystal packing reveals a second triubiquitin chain on the other side of the ABIN2-UBAN dimer (Fig. 5B). Thus, high local concentrations of linear ubiquitin chains might lead to the formation of a complex in which two linear ubiquitin chains bind either side of a ABIN2-UBAN dimer. It would be important to understand how triubiquitin chains may support oligomerization of UBAN-containing proteins like ABIN-2. For instance, diubiquitin versus triubiquitin chain binding to ABIN-2 might trigger differential readouts in downstream signaling activation, since only the latter would be predicted to induce oligomerization.

The **TNIP1** gene encoding ABIN-1 is associated with susceptibility to psoriasis in humans [55]. Moreover, genome-wide association studies (GWAS) reveal links between **TNIP1** mutations and systemic lupus erythematosus (SLE) [56,57] and asthma [58]. Finally, recurrent somatic mutations in the UBAN domain of ABIN-1 were found in Diffuse large B-cell lymphoma (DLBCL) (Fig. 4B, Table 1) [59], suggesting an involvement of linear ubiquitin chains.

**OPTINEURIN-UBAN**

The “Optic Neuropathy-Inducing” (OPTINEURIN)-UBAN domain was initially predicted by bioinformatic analysis together with other UBANs in ABIN proteins and NEMO (Fig. 4C) [54]. A structural study of OPTINEURIN-UBAN in complex with linear tetraubiquitins revealed a binding mode similar to that of linear ubiquitin with NEMO and ABIN-2 [23]. In this crystal structure, two tetraubiquitin chains within neighboring asymmetric units interact with opposing faces of an UBAN dimer, with two ubiquitins from each chain interacting with one face of a UBAN dimer (Fig. 5D). OPTINEURIN is a multifunctional protein,
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involved in protein trafficking by vesicles, autophagy and signal transduction. OPTINEURIN functions as an autophagy receptor to regulate selective autophagy at least partially through its UBAN domain, and its "microtubule associated protein 1 light chain 3 (LC3)-interacting region" (LIR) (Fig. 3B, Fig. 4C) [60]. Furthermore, the OPTINEURIN-UBAN/linear ubiquitin chain interaction regulates the selective autophagy pathway through "TANK Binding Kinase 1" (TBK1) kinase-dependent phosphorylation [35]. OPTINEURIN is also a negative regulator of the NF-κB signaling cascade (Fig. 3A).

Some of the patients suffering from glaucoma or amyotrophic lateral sclerosis (ALS) display mutations in OPTINEURIN (OPTN). A Glu478Gly mutation within the UBAN domain of OPTINEURIN (Fig. 4C, Fig. 5D, Table 1) [61], which recapitulates a mutation in ALS patients, disrupts its interaction with linear tetraubiquitin chains and abolishes OPTINEURIN-mediated inhibition of NF-κB signaling [23]. Together, these data suggest that linear ubiquitin chain recognition by OPTINEURIN is important for human health.

The UBAN domains in NEMO, ABINs and OPTINEURIN (Fig. 4A–C) display a similar interaction mode with linear ubiquitin chains (Fig. 5A–D), yet they have either positive or negative roles in TNF-induced immune signal regulation (Fig. 3A). It will be of interest to determine how their specific UBAN-dependent interacting partners and biological functions are precisely regulated to activate or inhibit the downstream signaling cascade.

The zinc fingers (ZFs)

**HOIL-1L-NZF**

HOIL-1L is a component of the LUBAC complex responsible for linear ubiquitination [62]. HOIL-1L itself is not considered to have the central catalytic ‘center’ of LUBAC, but HOIP, for generating linear ubiquitin chains. Linear ubiquitin-binding by HOIL-1L is mediated by its “Npl4 zinc finger” (NZF) domain and its C-terminal α-helical tail extension, referred to as the NZF-tail (Fig. 4D) [63]. The two domains are linked via a loop region, which itself does not interact with the ubiquitin chain (Fig. 5E) [63]. The NZF domain binds the canonical Ile44 hydrophobic surface on the distal ubiquitin within a diubiquitin chain, whereas the NZF tail binds the Phe4 surface of the proximal ubiquitin (Fig. 5E). Unlike the UBAN domains, HOIL-1L does not make extensive interactions with the C-terminal tail of the distal ubiquitin. Thus, the specificity of HOIL-1L-NZF for linear ubiquitin chains appears to be determined by the relative spatial orientation and the spacing between the distal and proximal ubiquitins. The physiological relevance of the HOIL-1L NZF is not fully understood besides its implication in NF-κB activation [63]. The interaction between linear ubiquitin chains and the HOIL-1L-NZF might be required for LUBAC-dependent linear ubiquitination. Alternatively, this interaction may protect chains from DUB-dependent hydrolysis, resulting in positive regulation of downstream signaling cascades. Furthermore, HOIL-1L might be recruited to the TNFR complex via the NZF domain.

As part of LUBAC, HOIL-1L regulates immune signaling cascades as well as selective autophagy by linearly ubiquitinating key signaling components, namely NEMO and RIPK2 (Fig. 3A). HOIL-1L NZF mutants that no longer interact with linear ubiquitin chains cannot fully activate NF-κB signaling in gene reporter assays [63], strongly suggesting that binding between HOIL-1L and linear ubiquitin chains is critical for regulation of the NF-κB signaling pathway. Mutations in the RBCK1 gene encoding HOIL-1L have been identified in patients suffering from polyglucosan body myopathy with immunodeficiency (Fig. 4D, Table 1) [64–66].

**A20-ZF7**

A20 protein was initially found to regulate NF-κB and cell death signaling pathways [67]. A20 harbors multiple functionally distinct domains that are important for regulating ubiquitin-dependent signaling cascades (Fig. 4E). For instance, A20 was first shown to acts as a ubiquitin E3 ligase to target “Receptor Interacting Protein Kinase 1” (RIPK1) for proteasomal degradation, and also as a DUB to hydrolyze Lys63-linked ubiquitin chains on RIPK1 within TNFR1 and RIPK1 within a signaling complex (Fig. 3A) [72]. A20 ZF7 simultaneously binds the Ile44 hydrophobic patch on the distal ubiquitin within diubiquitin, and forms a hydrogen bonding network with a region on the α-helix of proximal ubiquitin, consisting of residues Gln31/Asp32 and the backbones of residues Ala28/Gly35 (Fig. 5F).
Moreover, Leu71, Arg72, and Leu73 from the C-terminal tail of distal ubiquitin interact with A20 ZF7. Mutations in the linear ubiquitin-binding A20-ZF7 domain were found to be associated with B cell lymphomas (Fig. 4E, Table 1) [71,73–75]. Mutations within the catalytic “Ovarian Tumor” (OTU) domain and the ZF4 domain of A20 have been identified in patients with autoinflammatory syndrome, Hodgkin’s and non-Hodgkin’s lymphoma, and diabetes [73,76,77].

UBAN and ZF domains use distinct mechanisms to interact with linear ubiquitin chains, however, they both recognize the same surface on the distal ubiquitin within a linear ubiquitin chain, suggesting that one

| Table 1. Mutations identified in human genes encoding linear ubiquitin chain-binding domains. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Protein | Domain | Mutation (Nucleotide) | Mutation (Amino acid) | Associated disease |
| NEMO/IKKγ | UBAN | c.863C > G | p.A288G | Missense | Anhidrotic ecdermal dysplasia with immune deficiency (EDA-ID) |
| ABIN-1 | UBAN | c.2015G > A | p.E476K | Missense | Gastrointestinal diffuse large B cell lymphoma (DBLCL) |
| Optineurin | UBAN | c.1743A > G | p.E478G | Missense | Amyotrophic Lateral Sclerosis (ALS) |
| HOIL-1L | NZF | c.1160A > G | p.N387S | Nonsense, deletion | Invasive bacterial infections, systemic autoinflammation, amylopectinosis |
| A20 | ZF7 | c.2666G > C | p.P766P | Synonymous codon | Autoimmune disease |
| OTULIN | OTU | c.517delC | p.G174Dfs*2 | Missense, premature stop codon | Autoinflammation, panniculitis, dermatosis syndrome |
| CYLD | USP | c.1776delA | p.G593Asfs/p. K603X | Frameshift mutation | Familial cylindromatosis |
| | | c.1787G > A | p.G596D | Missense | Multiple familial trichoepithelioma (MFT) |
| | | c.1893_1906del ATATTATAGTAAAA | p.E631_ T636DfsX10 | 14 bp deletion, premature stop codon | Familial cylindromatosis |
| | | c.2172delA | p.K734X | Frameshift, premature stop codon | Brooke-Spiegler syndrome |
| | | c.2240A > G | p.E747G | Missense, premature stop codon | Brooke-Spiegler syndrome |
| | | c.2241_2242delAG | p.E747fsX763 | Missense | Multiple familial trichoepithelioma (MFT) |
| | | c.2272C > T | p.R758X | Nonsense | Familial cylindromatosis |

For A20, R= sequence variation present in non tumor cells suggesting a polymorphism [73].
linear ubiquitin chain can be captured by only one linear UBD at a time. The physiological relevance of linear ubiquitin chain recognition by the UBAN or the ZF domains in the immune responses and in other biological functions needs to be further clarified in the future.

The DUB catalytic domains for linear ubiquitin chains

**OTULIN-OTU**

Although the E3 ligase complex LUBAC specific for linear ubiquitination was discovered in 2006 [62], linear ubiquitin chain-specific DUBs were only recently discovered in 2013 [43,44]. The catalytic domain within the DUB OTULIN recognizes the linear ubiquitin dimer and specifically hydrolyzes linear ubiquitin chains (Fig. 4F and 5G) [43,44]. OTULIN binds to distinct surfaces on distal and proximal ubiquitins within diubiquitin, and specifically recognizes residues at the C-terminal tail of the distal ubiquitin that link the two ubiquitin molecules [43]. Whereas the distal ubiquitin uses its Ile44 hydrophobic surface for binding, the proximal ubiquitin binds the OTU domain via its α-helix and Phe4-centered surface (Fig. 5F). Importantly, Glu16 from the proximal ubiquitin takes part in the activation of OTULIN, revealing a substrate-assisted catalysis mechanism [27].

OTULIN negatively regulates various signaling cascades in which linear ubiquitination plays a role, such as immune signaling cascades and the cell death pathway mediated by the “TNF Receptor” (TNFR) or “Nucleotide-binding Oligomerization Domain-containing protein 2” (NOD2) (Fig. 3A). OTULIN is also known to directly interact with HOIP [78–80]. Interestingly, loss-of-function OTULIN mutations in mice lead to embryonic death between E12.5-E14 due to angiogenic deficits, indicating a role for OTULIN in vascularization [44].

Mutations within the catalytic OTU domain in OTULIN were identified in autoinflammation and cylindromatosis syndrome patients (Fig. 4F, Table 1) [81,82]. The autoimmune disease-related OTULIN mutations Leu272Pro and Tyr244Cys are not directly involved in interactions with linear ubiquitin chains but they are located on the helix arm that forms part of the binding pocket for the distal ubiquitin [43] (Fig. 5G). The Leu272Pro mutation of OTULIN suppresses the DUB-mediated hydrolysis of linear ubiquitin chains *in vitro*, supporting the physiological relevance and molecular function of linear ubiquitin binding by OTULIN [81].

**CYLD-USP**

CYLD is a tumor suppressor DUB that utilizes its catalytic specific protease (USP) domain to recognize ubiquitin chains and regulate immune signaling cascades (Fig. 4G). CYLD displays dual specificity for, and thus hydrolyzes, linear and Lys63-linked ubiquitin chains [80,83]. In the crystal structure of CYLD-USP in complex with linear diubiquitin, distal ubiquitin uses a hydrophobic patch centered on Ile44, as well as its C-terminal tail including residues Val70-Gly76, to interact with the USP domain (Fig. 5H). The CYLD-USP binding region on proximal ubiquitin involves residues from the Phe4 patch (Fig. 4E). Moreover, similar to the OTU domain, Glu16 of the proximal ubiquitin is shown to contribute to the hydrogen bonding with the CYLD-USP domain and thus to the catalytic activity of the CYLD enzyme. CYLD functions as an inhibitor in the TNFR and NOD2 signaling cascades by deubiquitinating both linear and Lys63-linked ubiquitin chains, which are important for mediating these signaling cascades (Fig. 3A) [46]. Interestingly, CYLD makes a signaling complex with HOIP via “Spermatogenesis Associated 2” (SPATA2) at TNFR [84–87]. The interplay between the deubiquitinase CYLD or OTULIN and the ubiquitin E3 ligase HOIP in a complex is an interesting aspect to consider for the regulation of downstream signaling cascades. Several mutations in CYLD, mostly within the USP domain, have been identified in patients with familial cylindromatosis (Fig. 4G, Table 1) [88–93].

Although OTULIN and CYLD have overlapping functions in the regulation of immune signaling cascades (Fig. 3A), their roles *in vivo* seem to be distinct based not only on their association with different human diseases but also on the distinct phenotypes of their genetically modified mouse models. *Cyld<sup>+/−</sup>* mice show no gross defects and are born at the expected Mendelian ratios [94], whereas OTULIN loss-of-function mutant mice (Gumby mice) are embryonic lethal [44]. Their distinct functions in biology likely depend on their different noncatalytic functions and interacting partners, as well as the regulation of their expression and activity.

**Concluding remarks**

The first linear ubiquitin chain-specific UBD was discovered in NEMO and shown to regulate the inflammatory NF-κB signaling cascade [19]. Since that discovery, roles for linear ubiquitination have been demonstrated in various diverse signaling pathways. Thus far, all proteins with linear ubiquitin chain
binding domains have been established as critical regulators of immune-related signaling cascades. Many gene mutations associated with autoimmune diseases are within linear ubiquitin chain-specific UBDs or DUB catalytic domains, clearly indicating a role for disrupted linear ubiquitination in human disease.

**Outstanding questions**

- Do linear ubiquitin chain-specific UBDs equally recognize free ubiquitin chains and substrate-conjugated ubiquitin chains?
- What are the contributions of ubiquitin-UBD interaction in enzymatic complexes? For example, does the HOIL-1L-NZF interaction with linear ubiquitin chains regulate LUBAC catalytic activity? Does the NEMO-UBAN interaction with linear ubiquitin chains affect the activity of the IKK kinases? Do these interactions have an allosteric effect?
- Does the interaction of the linear UBDs with ubiquitin chains protect them from hydrolysis mediated by OTULIN or CYLD? Does this interaction regulate linear ubiquitin chain length?
- Are there unknown UBDs that specifically recognize linear-Lys linked mixed chains, or post-translationally modified linear ubiquitin chains?

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**Author contributions**

LMF, SR, and FI wrote the manuscript and made figures and a table.

**References**

1. Catic A & Ploegh HL (2005) Ubiquitin-conserved protein or selfish gene? *Trends Biochem Sci* **30**, 600–604.
2. Hershko A & Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* **67**, 425–479.
3. Dubiel W & Gordon C (1999) Ubiquitin pathway: another link in the polyubiquitin chain? *Curr Biol* **9**, R554–R557.
4. Wagner SA, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M & Choudhary C (2011) A proteome-wide, quantitative survey of *in vivo* ubiquitylation sites reveals widespread regulatory roles. *Mol Cell Proteomics* **10**, M111013284.
5. Asaoka T & Ikeda F (2015) New insights into the role of ubiquitin networks in the regulation of antiapoptosis pathways. *Int Rev Cell Mol Biol* **318**, 121–158.
6. Swatek KN & Komander D (2016) Ubiquitin modifications. *Cell Res* **26**, 399–422.
7. Yamano K, Matsuda N & Tanaka K (2016) The ubiquitin signal and autophagy: an orchestrated dance leading to mitochondrial degradation. *EMBO Rep* **17**, 300–316.
8. Harper JW, Orduereau A & Heo JM (2018) Building and decoding ubiquitin chains for mitophagy. *Nat Rev Mol Cell Biol* **19**, 93–108.
9. Ikeda F, Crosetto N & Dikic I (2010) What determines the specificity and outcomes of ubiquitin signaling? *Cell* **143**, 677–681.
10. Ebner P, Versteeg GA & Ikeda F (2017) Ubiquitin enzymes in the regulation of immune responses. *Crit Rev Biochem Mol Biol* **52**, 425–460.
11. Ohtake F, Saeki Y, Ishido S, Kanno J & Tanaka K (2016) The K48-K63 branched ubiquitin chain regulates NF-kappaB signaling. *Mol Cell* **64**, 251–266.
12. Sagar GD, Gereben B, Callebaut I, Mormon JP, Zeold A, da Silva WS, Luongo C, Dentice M, Tente SM, Freitas BC *et al.* (2007) Ubiquitination-induced conformational change within the deiodinase dimer is a switch regulating enzyme activity. *Mol Cell Biol* **27**, 4774–4783.
13. Bowman J, Rodgers MA, Shi M, Amatya R, Hostager B, Iwai K, Gao SJ & Jung IU (2015) Posttranslational modification of HOIP blocks toll-like receptor 4-mediated linear-ubiquitin-chain formation. *MBio* **e01777-15**.
14. Cohen A, Rosenthal E & Shifman JM (2017) Analysis of structural features contributing to weak affinities of ubiquitin/protein interactions. *J Mol Biol* **429**, 3353–3362.
15. Dikic I, Wakatsuki S & Walters KJ (2009) Ubiquitin-binding domains - from structures to functions. *Nat Rev Mol Cell Biol* **10**, 659–671.
16. Husnjak K & Dikic I (2012) Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. *Annu Rev Biochem* **81**, 291–322.
17. Hauenstein AV, Xu G, Kabaleeswaran V & Wu H (2017) Evidence for M1-linked polyubiquitin-mediated conformational change in NEMO. *J Mol Biol* **429**, 3793–3800.
18. Dziedzic SA, Su Z, Jean Barrett V, Najafov A, Moenkhtaa AK, Amin P, Han H, Sun L, Zhu H, Ma A *et al.* (2017) ABIN-1 regulates RIPK1 activation by linking Met1 ubiquitylation with Lys63 deubiquitylation in TNF-RSC. *Nat Cell Biol* **20**, 58–68.
19. Rahighi S, Ikeda F, Kawasaki M, Akutsu M, Suzuki N, Kato R, Kenschte T, Uejima T, Bloor S, Komander
Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. 

Wauer T, Swatek KN, Wagstaff JL, Gladkova C, Pruneda JN, Michel MA, Gersch M, Johnson CM, Freund SM & Komander D (2015) Ubiquitin Ser65 phosphorylation affects ubiquitin structure, chain assembly and hydrolysis. 

Jabusch JR & Deutsch HF (1985) Localization of lysines acylated in ubiquitin reacted with p-nitrophenyl acetate. 

Ohtake F, Saeki Y, Sakamoto K, Ohtake K, Nishikawa H, Tsuchiya H, Ohta T, Tanaka K & Kanno J (2015) Ubiquitin acetylation inhibits polyubiquitin chain elongation. 

Nakazawa S, Okawa D, Ishii R, Ayaki T, Takahashi Ohtake F, Saeki Y, Sakamoto K, Ohtake K, Nishikawa Kawakami H, Takeda H, Ishitani R, Kamei K, Takeyoshi I, Kawakami H et al. (2016) Linear ubiquitination is specific deubiquitinase gumby regulates angiogenesis. 

Ikeda F, Deribe YL, Skanland SS, Stiegltz B, Grabbe C, Franz-Wachtel M, van Wijk SJ, Goswami P, Nagy V, Terzic J et al. (2011) SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. 

Tokunaga F, Nakagawa T, Nakahara M, Saeki Y, Taniguchi M, Sakata S, Tanaka K, Nakano H & Iwai K (2011) SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. 

Gerlach B, Cordier SM, Schmukle AC, Emmerich CH, Rieser E, Haas TL, Webb AI, Rickard JA, Anderton H, Wong WW et al. (2011) Linear ubiquitination prevents inflammation and regulates immune signalling. 

Smit JJ & Sixma TK (2014) RBR E3-ligases at work. 

Stiegltz B, Morris-Davies AC, Koliopoulos MG, Christodoulou E & Rittinger K (2012) LUBAC synthesizes linear ubiquitin chains via a thioester intermediate. 

Keusekotten K, Elliott PR, Glockner L, Fiil BK, Damgaard RB, Kulathu Y, Wauer T, Hospenthal MK, Gyrd-Hansen M, Krappmann D et al. (2013) OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. 

Rivkin E, Almeida SM, Ceccarelli DF, Huang YC, MacLean TA, Srikumar T, Huang H, Dunham WH, Fukushima R, Xie G et al. (2013) The linear ubiquitin-specific deubiquitinase gumby regulates angiogenesis. 

Sato Y, Goto E, Shibata Y, Kubota Y, Yamagata A, Goto-Ito S, Kubota K, Inoue J, Takekawa M,
Tokunaga F et al. (2015) Structures of CYLD USP with Met1- or Lys63-linked diubiquitin reveal mechanisms for dual specificity. *Nat Struct Mol Biol* **22**, 222–229.

46 Hrdinka M, Fiil BK, Zucca M, Leske D, Bagola K, Yabal M, Elliott PR, Damgaard RB, Komander D, Jost PJ et al. (2016) CYLD Limits Lys63- and Met1-Linked ubiquitin at receptor complexes to regulate innate immune signaling. *Cell Rep* **14**, 2846–2858.

47 Wagner S, Carpentier I, Rogov V, Kreike M, Ikeda F, Lohr F, Wu CJ, Ashwell JD, Dotsch V, Dikic I, et al. (2008) Ubiquitin binding mediates the NF-kappaB inhibitory potential of ABIN proteins. *Oncogene* **27**, 3739–3745.

48 Yoshikawa A, Sato Y, Yamashita M, Mimura H, Yamagata A & Fukai S (2009) Crystal structure of the NEMO ubiquitin-binding domain in complex with Lys 63-linked di-ubiquitin. *FEBS Lett* **583**, 3317–3322.

49 Lo YC, Lin SC, Rospigliosi CC, Conze DB, Wu CJ, Ashwell JD, Eliezer D & Wu H (2009) Structural basis for recognition of diubiquitins by NEMO. *Mol Cell* **33**, 602–615.

50 Vincendeau M, Hadian K, Messias AC, Brenke JK, Halander J, Griesbach R, Groczmiel U, Bertossi A, Stehle R, Nagel D et al. (2016) Inhibition of canonical NF-kappaB signaling by a small molecule targeting NEMO-ubiquitin interaction. *Sci Rep* **6**, 18934.

51 Döffinger R, Smahi A, Bessia C, Geissmann F, Feinberg A, Durandy A, Bodemer C, Kenrick S, Dupuis-Girod S, Blanche S et al. (2001) X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kB signaling. *Nat Genet* **27**, 277.

52 Hubeau M, Ngadjeu F, Puel A, Israel L, Feinberg J, Chrabieh M, Belani K, Bodemer C, Fabre I, Plebani A et al. (2011) New mechanism of X-linked anhidrotic ectodermal dysplasia with immunodeficiency: impairment of ubiquitin binding despite normal folding of NEMO protein. *Blood* **118**, 926–935.

53 Filipe-Santos O, Bustamante J, Haverkamp MH, Vinolo E, Ku C-L, Puel A, Frucht DM, Christel K, von Bernuth H, Jouanguy E et al. (2006) X-linked susceptibility to mycobacteria is caused by mutations in NEMO impairing CD40-dependent IL-12 production. *J Exp Med* **203**, 1745–1759.

54 Verstrepen L, Carpentier I & Beyaert R (2014) The biology of A20-binding inhibitors of NF-kappaB activation (ABINs). *Adv Exp Med Biol* **809**, 13–31.

55 Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, Gudjonsson JE, Li Y, Tejasvi T, Feng BJ et al. (2009) Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* **41**, 199–204.

56 Han JW, Zheng HF, Cui Y, Sun LD, Ye DQ, Hu Z, Xu JH, Cai ZM, Huang W, Zhao GP et al. (2009) Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet* **41**, 1234–1237.

57 Gateva V, Sandling JK, Hom G, Taylor KE, Chung SA, Sun X, Ortmann W, Kosoy R, Ferreira RC, Nordmark G et al. (2009) A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* **41**, 1228–1233.

58 Li X, Amberlford EJ, Howard TD, Moore WC, Torgerson DG, Li H, Busse WW, Castro M, Erzurum SC, Israel E et al. (2012) Genome-wide association studies of asthma indicate opposite immunopathogenesis direction from autoimmune diseases. *J Allergy Clin Immunol* **130**, 861–868. e7.

59 Dong G, Chanudet E, Zeng N, Appert A, Chen Y-W, Au W-Y, Hamoudi RA, Watkins AJ, Ye H, Liu H et al. (2011) A20, ABIN-1/2, and CARD11 mutations and their prognostic value in gastrointestinal diffuse large b-cell lymphoma. *Clin Cancer Res* **17**, 1440–1451.

60 Boyle KB & Randow F (2013) The role of ‘eat-me’ signals and autophagy cargo receptors in innate immunity. *Curr Opin Microbiol* **16**, 339–348.

61 Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, Kinoshita Y, Kamada M, Nodera H, Suzuki H et al. (2010) Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* **465**, 223.

62 Kirisako T, Kamei K, Murata S, Kato M, Fukushima H, Kanie M, Sano S, Tokunaga F, Tanaka K & Iwai K (2006) A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J* **25**, 4877–4887.

63 Sato Y, Fujita H, Yoshikawa A, Yamashita M, Yamagata A, Kaiser SE, Iwai K & Fukai S (2011) Specific recognition of linear ubiquitin chains by the Npl4 zinc finger (NZF) domain of the HOIL-1L subunit of the linear ubiquitin chain assembly complex. *Proc Natl Acad Sci USA* **108**, 20520–20525.

64 Boisson B, Laplantine E, Prando C, Giliani S, Israëlsson E, Xu Z, Abhyankar A, Israel L, Trevejo-Nunez G, Bogunovic D et al. (2012) Immunodeficiency, autoinflammation and amylopectinosis in humans with inherited HOIL-1 and LUBAC deficiency. *Nat Immunol* **13**, 1178–1186.

65 Wang K, Kim C, Bradfield J, Guo Y, Tосska Е, Otieno FG, Hou C, Thomas K, Cardinale C, Lyon GJ et al. (2012) Polyglucosan body myopathy caused by defective ubiquitin ligase RBCK1. *Am J Hum Genet* **88**, 465–477.

66 Nilsson J, Schoser B, Laforet P, Kalev O, Lindberg C, Romero NB, Dávila López M, Akman HO, Wahi B, Iglseider S et al. (2013) Polyglucosan body myopathy caused by defective ubiquitin ligase RBCK1. *Ann Neurol* **74**, 914–919.
67 Lork M, Verhelst K & Beyaert R (2017) CYLD, A20 and OTULIN deubiquitinases in NF-kappaB signaling and cell death: so similar, yet so different. *Cell Death Differ* **24**, 1172–1183.

68 Wertz IE, O’Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL et al. (2004) De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signaling. *Nature* **430**, 694–699.

69 Shembade N, Parvatiyar K, Harhaj NS & Harhaj EW (2009) The ubiquitin-editing enzyme A20 requires RNF11 to downregulate NF-kappaB signalling. *EMBO J* **28**, 513–522.

70 Verhelst K, Carpentier I, Kreike M, Meloni L, Verstrepen L, Kensch T, Dikic I & Beyaert R (2012) A20 inhibits LUBAC-mediated NF-kappaB activation by binding linear polyubiquitin chains via its zinc finger 7. *EMBO J* **31**, 3845–3855.

71 Tokunaga F, Nishimatsu H, Ishitani R, Goto E, Noguchi T, Mio K, Kamei K, Ma A, Iwai K & Nureki O (2012) Specific recognition of linear polyubiquitin by A20 zinc finger 7 is involved in NF-kappaB regulation. *EMBO J* **31**, 3856–3870.

72 Draber P, Kupka S, Reichert M, Draberova H, Lafont E, de Miguel D, Spilgies L, Surinova S, Taraborrelli L, Hartwig T et al. (2015) LUBAC-recruited CYLD and A20 regulate gene activation and cell death by exerting opposing effects on linear ubiquitin in signaling complexes. *Cell Rep* **13**, 2258–2272.

73 Schmitz R, Hansmann ML, Bohle V, Martin-Subero JI, Hartmann S, Mechtlersheimer G, Klapper W, Vater I, Giefing M, Gesk S et al. (2009) TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma. *J Exp Med* **206**, 981–989.

74 Musone SL, Taylor KE, Niiittham J, Chu C, Poon A, Liao W, Lam ET, Ma A, Kwok PY & Criswell LA (2011) Sequencing of TNFAIP3 and association of genetic polymorphisms in TNFAIP3 mediate risk for autoimmunity. *Genes Cells* **16**, 1215–1230. e20

75 Lodolce JP, Kolodziej LE, Rhee L, Kariuki SN, Franke BS, McGreal NM, Logsdon MF, Bartulis SJ, Perera MA, Ellis NA et al. (2010) African-derived genetic polymorphisms in TNFAIP3 mediate risk for autoimmunity. *J Immunol* **184**, 7001–7009.

76 Catrysse L, Vereecke L, Beyaert R & van Loo G (2014) A20 in inflammation and autoimmunity. *Trends Immunol* **35**, 22–31.

77 Vereecke L, Beyaert R & van Loo G (2009) The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. *Trends Immunol* **30**, 383–391.

78 Schaeffer V, Akutsu M, Olma MH, Gomes LC, Kawasaki M & Dikic I (2014) Binding of OTULIN to the PUB domain of HOIP controls NF-kappaB signaling. *Mol Cell* **54**, 349–361.

79 Elliott PR, Nielsen SV, Marco-Casanova P, Fiil BK, Keusekotten K, Mailand N, Freund SM, Gyrd-Hansen M & Komander D (2014) Molecular basis and regulation of OTULIN-LUBAC interaction. *Mol Cell* **54**, 335–348.

80 Takiuchi T, Nakagawa T, Tamiya H, Fujita H, Sasaki Y, Saeki Y, Takeda H, Sawasaki T, Buchberger A, Kimura T et al. (2014) Suppression of LUBAC-mediated linear ubiquitination by a specific interaction between LUBAC and the deubiquitinases CYLD and OTULIN. *Genes Cells* **19**, 254–272.

81 Damgaard RB, Walker JA, Marco-Casanova P, Morgan NV, Titheradge HL, Elliott PR, McHale D, Maher ER, McKenzie ANJ & Komander D (2016) The deubiquitinase OTULIN is an essential negative regulator of inflammation and autoimmunity. *Cell* **166**, 1215–1230. e20

82 Zhou Q, Yu X, Demirkaya E, Deutich N, Stone D, Tsai WL, Kuehn HS, Wang H, Yang D, Park YH et al. (2016) Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease. *Proc Natl Acad Sci USA* **113**, 10127–10132.

83 Asaoka T, Almagro J, Ehrhardt C, Tsai I, Schleiffer A, Deszcz L, Juntilla S, Ringrose L, Mechtler K, Kavirayani A et al. (2016) Linear ubiquitination by LUBEL has a role in Drosophila heat stress response. *EMBO Rep* **17**, 1624–1640.

84 Wagner SA, Satpathy S, Beli P & Choudhary C (2016) SPATA2 links CYLD to the TNF-alpha receptor signaling complex and modulates the receptor signaling outcomes. *EMBO J* **35**, 1868–1884.

85 Schlicher L, Wissler M, Preiss F, Brauns-Schubert P, Jakob C, Dumit V, Borner C, Dengjel J & Maurer U (2016) SPATA2 promotes CYLD activity and regulates TNF-induced NF-kappaB signaling and cell death. *EMBO Rep* **17**, 1485–1497.

86 Kupka S, De Miguel D, Draber P, Martino L, Surinova S, Rittinger K & Walczak H (2016) SPATA2 links CYLD to the TNF-alpha receptor signaling complex and modulates the receptor signaling outcomes. *EMBO J* **35**, 1485–1497.

87 Elliott PR, Leske D, Hrdinka M, Bagola K, Fiil BK, Keusekotten K, Mailand N, Freund SM, Gyrd-Hansen M & Komander D (2014) Molecular basis and regulation of OTULIN-LUBAC interaction. *Mol Cell* **54**, 335–348.

88 Bignell GR, Warren W, Seal S, Takahashi M, Rapley E, Barfoot R, Green H, Brown C, Biggs PJ, Lakhani SR et al. (2000) Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet* **25**, 160.
89 Zuo YG, Xu Y, Wang B, Liu YH, Qu T, Fang K & Ho MG (2007) A novel mutation of CYLD in a Chinese family with multiple familial trichoepithelioma and no CYLD protein expression in the tumour tissue. Br J Dermatol 157, 818–821.

90 Scheinfeld N, Hu G, Gill M, Austin C & Çelebi JT (2003) Identification of a recurrent mutation in the CYLD gene in Brooke–Spiegler syndrome. Clin Exp Dermatol 28, 539–541.

91 Nasti S, Pastorino L, Bruno W, Gargiulo S, Battistuzzi L, Zavattaro E, Leigheb G, De Francesco V, Tulli A, Mari F et al. (2009) Five novel germline function-impairing mutations of CYLD in Italian patients with multiple cylindromas. Clin Genet 76, 481–485.

92 Hu G, Önder M, Gill M, Aksakal B, Öztas M, Ali Gürer M & Çelebi JT (2003) A novel missense mutation in CYLD in a family with Brooke–Spiegler syndrome. J Invest Dermatol 121, 732–734.

93 Liang YH, Gao M, Sun LD, Liu LJ, Cui Y, Yang S, Fan X, Wang J, Xiao FL & Zhang XJ (2005) Two novel CYLD gene mutations in Chinese families with trichoepithelioma and a literature review of 16 families with trichoepithelioma reported in China. Br J Dermatol 153, 1213–1215.

94 Reiley WW, Zhang M, Jin W, Losiewicz M, Donohue KB, Norbury CC & Sun SC (2006) Regulation of T cell development by the deubiquitinating enzyme CYLD. Nat Immunol 7, 411–417.