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Ubiquitin binding domains — from structures to functions

Ivan Dikic[1,2,3,*], **Soichi Wakatsuki**[4], **Kylie J. Walters**[5]

[1] Institute of Biochemistry II and Cluster of Excellence Macromolecular Complexes, Goethe University Frankfurt,Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany

[2] Tumor Biology Program, Mediterranean Institute for Life Sciences, 21000 Split, Croatia

[3] Department of Immunology, School of Medicine, University of Split, Soltanska 2, 21 000 Split, Croatia

[4] Structural Biology Research Center, Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK), Tsukuba, Ibaraki 305-0801, Japan

[5] Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA

Preface

Ubiquitin-binding domains (UBDs) are modular elements that bind non-covalently to the protein modifier ubiquitin. Recent atomic-level resolution structures of ubiquitin–UBD complexes have revealed some of the mechanisms that underlie the versatile functions of ubiquitin *in vivo*. The preferences of UBDs for ubiquitin chains of specific length and linkage are central contributors to these functions. These preferences originate from multimeric interactions, whereby UBDs synergistically bind multiple ubiquitin subunits, and from contacts with regions that link ubiquitin molecules into a polymer. The sequence context of UBDs and the conformational changes that follow their binding to ubiquitin also contribute to ubiquitin signalling. The new structure-based insights provide strategies for controlling cellular processes by targeting ubiquitin–UBD interfaces.

Correspondence ID: ivan.dikic@biochem2.de, Phone: +49-69-6301 83647.

Online summary

- Ubiquitin is an intracellular signalling molecule that is conjugated to a wide spectrum of proteins. Its conjugation to itself yields Lys or Met-conjugated chains, thus expanding its repertoire of signalling networks.
- Ubiquitin binding domains (UBDs) are modular elements that bind non-covalently to the protein modifier ubiquitin.
- Specific ubiquitin–UBD interactions are crucial for the regulation of multiple cellular functions including protein stability, receptor trafficking, DNA damage responses and inflammatory pathways.
- UBD preferences for distinct ubiquitin chains of specific length and linkage are mediated via multimeric interactions, sequence context of the UBD and conformational changes following binding.
- Structures of ubiquitin–UBD complexes have revealed mechanisms of selectivity and specificity in their functional interactions in *vivo*.
- Defects in ubiquitin–UBD interactions are relevant for disease development like inflammation and cancer. The new structure-based insights provide strategies for a design of novel approaches that can therapeutically target ubiquitin–UBD interaction surfaces.

Ubiquitin is a cellular signal that labels proteins in a highly controlled manner. Conjugation of ubiquitin to a target protein or to itself is regulated by the sequential activity of ubiquitin activating (E1), conjugating (E2) and ligating (E3) enzymes, and typically results in the addition of a ubiquitin moiety to either the ε-amino group of a Lys residue, or to the extreme amino terminus of a polypeptide- (FIG. 1). Ubiquitin chains can grow as E2 and/or E3 enzymes catalyse the formation of an isopeptide bond between a carboxyl group of ubiquitin Gly76 and an ε amino group of another ubiquitin's Lys. There are seven Lys residues in ubiquitin (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63), allowing for seven possible homotypic linkage types and multiple layers of possible heterotypic chains. As a consequence, cellular proteins are modified by a variety of ubiquitin signals: monoubiquitin, multiple monoubiquitin or ubiquitin chains, which can be of diverse length and linkage.

Whereas the addition of a single ubiquitin to a target protein (monoubiquitylation) can alter protein activity and localization (regulating endocytosis, lysosomal targeting, meiosis and chromatin remodelling), the formation of a diverse array of ubiquitin chains (polyubiquitylation) is implicated in events such as proteasomal targeting, immune signalling pathways and DNA repair. The newest addition to ubiquitin polymers includes linear (head-to-tail) ubiquitin chains, which are assembled by a specific ligase complex called LUBAC (linear ubiquitin chain assembly complex), and are crucial for the nuclear factor-κB (NF-κB) signalling-.

In these processes, ubiquitin acts as a signalling component capable of triggering molecular events in cells. Ubiquitin does so by operating as a reversible and highly versatile regulatory signal for an expanding number of ubiquitin-binding domains (UBD) present in cellular proteins. Many molecular details of signal transmission from ubiquitylated proteins (modified substrates following different cellular stimuli) toward effector proteins (ubiquitin receptors containing one or more UBDs) have been traced in the last decade (FIG. 2).

In this Review, we discuss how different ubiquitin signals can be recognized by distinct UBDs present in a multitude of cellular proteins (currently estimated to be more than 150). Different mechanisms have evolved to achieve *in vivo* specificity in the UBDs–ubiquitin interactions, including increased avidity (the combined synergistic strength of bond affinities during multifaceted interactions), distinct affinities for ubiquitin chains of specific linkage, the contribution by UBD-independent sequences and, finally, conformational changes following the UBDs–ubiquitin interactions. These factors create environments in which the effective affinity and selectivity of ubiquitin–UBD interactions are functionally relevant in the context of a living cell. We also provide several recent examples in which structural and functional analyses were essential for uncovering the molecular basis of cellular processes, such as the regulation of protein stability, receptor trafficking in the endosome, DNA damage responses and inflammatory pathways. Moreover, we describe defects in ubiquitin–UBD interactions that are relevant for the development of diseases such as inflammation and cancer, and elaborate on the design of novel therapeutic approaches that can target ubiquitin–UBD interaction surfaces.

Ubiquitin: a diverse cellular signal

Ubiquitylation is among the most widely used protein modifications involved in regulating cellular signalling and homeostasis. At the molecular level, ubiquitin can be viewed as a prototypic intracellular signal that is inducibly and reversibly attached to a wide spectrum of proteins and as such regulates a multitude of cellular functions. Ubiquitin contains a diverse surface architecture and forms differently-coupled ubiquitin chains thus expanding its capacity to act as a versatile signalling messenger.

Monoubiquitin —- one fold, many dynamic conformations

The canonical ubiquitin fold is formed by a five-stranded β-sheet, a short 3_{10} helix and a 3.5-turn α helix (FIG. 3a). The carboxyl-terminal tail of ubiquitin is exposed, which allows its covalent linkage to target proteins. Ubiquitin is a small protein of 76 amino acids with a surface area of only 4800 A. Most UBDs interact with a solvent-exposed hydrophobic patch, which includes Leu8, Ile44, and Val70 and is located in ubiquitin's β-sheet (FIG. 3a). Although most UBDs tend to target the same surface of ubiquitin, the amino acids that surround the hydrophobic patch are chemically diverse. As a consequence, the greater than two dozen UBDs characterized so far (TABLE 1) include a myriad of structural folds and unique binding modes.

X-ray and nuclear magnetic resonance (NMR) structures of ubiquitin bound to different UBDs indicate that it adopts slightly different conformations depending on its binding partner. Lange *et al.* used nuclear magnetic resonance residual dipolar couplings (RDCs) to analyse the full dynamic behaviour of ubiquitin on the picosecond to microsecond time scale in solution. They demonstrated that ubiquitin exhibits the structural heterogeneity of its UBD-bound states, as each corresponded to a member of the ensemble of structures sampled by free ubiquitin over time (FIG. 3b). This structural heterogeneity contributes strongly to the adaptive interface of ubiquitin during binding events to different UBDs.

Ubiquitin chains — diverse cellular signals

An attractive model that describes how the outcome of ubiquitylation is determined in cells proposed that the destiny of ubiquitylated substrates relies on the ubiquitin linkage type and chain length. This early model applies best to ubiquitin chains that are homogeneous, namely those assembled through a single linkage type. Structural studies have revealed significant differences between ubiquitin chains of Lys48 (FIG. 3c) and Lys63 (FIG. 3d)/ linear linkages (FIG. 3e). Lys63- and linearly-linked diubiquitins adopt similar extended conformations with no contact between the two ubiquitin moieties. By contrast, the ubiquitin moieties of Lys48-linked tetraubiquitin pack against each other, with exchange in solution between the closed, packed structure and an extended, opened one.

Importantly, structures of ubiquitin chains in complex with UBDs have revealed that Lys48-, Lys63- and linearly-linked ubiquitin molecules adopt a wide range of inter-moiety arrangements that depend on their binding partners (Supplementary information S1 (figure)). For example, the open conformation of Lys63-linked diubiquitin is stretched in the complex with the endosome-associated ubiquitin isopeptidase AMSH (associated molecule with the

SH3 domain of STAM) to allow efficient cleavage of the isopeptide bond. In contrast, Lys63-linked diubiquitin in complex with an antibody adopts a more compact conformation in which the two ubiquitin moieties are packed together with substantial surface contacts between them. These structural studies have led to the appreciation of ubiquitin chains as flexible modules that can be shaped by their cellular context.

UBDs — multiple folds for ubiquitin

UBDs are diverse modules within a larger protein that are able to bind, and often distinguish, different types of ubiquitin modifications (monoubiquitin and ubiquitin chains of different linkage type). The number of identified UBDs is constantly growing, with more than 20 different families to date (TABLE 1). UBDs diverge both in structure and their type of ubiquitin recognition. Most commonly, they fold into α-helical structures (FIG. 4a), zinc fingers (ZnFs) (FIG. 4b), the UBC (ubiquitin-conjugating) domain present in E2 enzymes (FIG. 4c) or plekstrin homology (PH) folds (FIG. 4d and 4e). Several comprehensive reviews have described the various classes of ubiquitin-binding domains and their biological properties-. In the following subsections, we discuss the principles by which distinct classes of UBDs interact with ubiquitin modifications.

UBDs that bind monoubiquitin

A large majority of UBDs uses α-helical structures to bind a hydrophobic patch within the β-sheet of ubiquitin. The ubiquitin-interacting motif (UIM), inverted UIM (MIU or IUIM) and ubiquitin-binding zinc finger (UBZ) bind this region of ubiquitin with a single helix-oriented either parallel or anti-parallel to the central β-strand (FIG. 4b and Supplementary information S2 (figure)). Other ubiquitin-binding elements, including (UBA) (ubiquitin-associated) (FIG. 4a) and CUE (coupling of ubiquitin conjugation to endoplasmic reticulum degradation) domains-, bind ubiquitin through two helices that are discontinuous. It is currently unclear why so many variations of helical structures have evolved to interact with ubiquitin and regulate its downstream signalling. Several examples of their involvement in specific ubiquitin signalling to regulate proteasomal functions, DNA damage response or receptor endocytosis are discussed below.

β-sheets present in different structural domains can also engage in recognition of monoubiquitin. For example, the β-sheet of E2 ubiquitin-conjugating enzyme UBCH5c interacts with ubiquitin's hydrophobic Ile44-containing surface (FIG. 4c). UBCH5c functions with the E3 ligase BRCA1 (Breast Cancer Gene 1), a tumour suppressor protein with breast cancer-associated mutations. This non-covalent interaction between UBCH5c and ubiquitin activates the self-assembly of ubiquitin chains on UBCH5c and in turn the processive ubiquitylation by BRCA1, which is crucial for coordinating DNA damage regulatory complexes (see below). Another example is the GLUE (GRAM-like ubiquitin-binding in EAP45) domain present in the EAP45 (ELL-associated protein of 45 kDa) subunit of the endocytic sorting complex ESCRT (endosomal sorting complexes required for transport). This domain folds into a split PH domain that also uses residues of β-strands S5 and S6 to bind the hydrophobic patch of ubiquitin; although in this case, additional residues from a loop and α-helix are used too, (FIG. 4d). The Pru (PH receptor for ubiquitin) domain

present in the proteasomal receptor Rpn13 (Regulatory Particle, Non-ATPase-like 13) binds to the β-strand surface of ubiquitin with a PH domain; however, it does so in an entirely different manner. Three loops within its ubiquitin-binding domain form the binding interface to contact a larger surface compared to EAP45 and to form hydrogen bonds with His68 in ubiquitin (FIG. 4e). These properties contribute to its higher affinity for monoubiquitin. Hence, even when the same structural domain is used to bind the Ile44-containing surface of ubiquitin, recognition can be through different UBD surfaces and structural elements. This finding highlights the difficulty of identifying UBDs and of predicting ubiquitin-binding surfaces without experimental data.

The common use of the Ile44-containing surface by UBDs causes their binding to monoubiquitin to be mutually exclusive, suggesting that in most cases, a ubiquitin moiety is only acted upon by one UBD. It is possible that this mutual exclusivity is an important component of effective ubiquitin signalling, as it can prevent each ubiquitin from stimulating multiple processes, which could lead to discordance within the cellular context. An exception to this rule is demonstrated by variations in the ZnF domains; these recognize monoubiquitin by binding to three different regions on its surface (FIG. 4b). The NPL4 (nuclear protein localization 4) ZnF (NZF) domain, which serves as an ubiquitin-binding adapter protein in the endoplasmic reticulum (ER)-associated degradation (ERAD) pathway, and the ubiquitin-binding Zn fingers (UBZ domains) present in translesion synthesis polymerases (TLS) bind to the β-sheet Ile44-containing hydrophobic surface of ubiquitin. By contrast, the RABEX-5 (Rab5 guanine nucleotide exchange factor) A20-type ZnF domain recognizes a polar surface of ubiquitin centred on Asp58, which empowers RABEX5 to act with ligase capacity toward trans substrates. A different binding surface is contacted by the deubiquitinase (DUB; also known as deubiquitylating or deubiquitinating enzyme) isopeptidase T (IsoT), which has a ZnF domain that binds to the C-terminal residues of ubiquitin. The unique ubiquitin binding mode of the IsoT ZnF domain ensures that it only disassembles unanchored chains and suggests that it could act in conjunction with other UBDs that bind to ubiquitin's Ile44-containing surface. The impressive diversity of DUB structure and ubiquitin recognition strategies was recently described in an excellent review article.

There is no evidence that binding affinity for ubiquitin can be predicted based on the UBD structural fold. By contrast, a larger range of affinities is found within the UBA domain family than between UBA domains and UBZ or CUE domains. There is also no clustering of particular UBD folds into specific effector proteins or to selective cellular functions. Rather, the same class of UBD, for example UBA domains, is found in a variety of proteins with numerous additional domains implicated in a wide spectrum of cellular functions that include endocytosis, proteasome signalling and apoptosis.

Lys linkage-specific UBDs

Linkage-specific ubiquitin recognition contributes to the diverse set of functional outcomes associated with ubiquitylation. The pioneering work of Cecile Pickart and colleagues revealed diversity in ubiquitin chain recognition preferences for 30 different UBA domains. Although some UBA domains showed little discretion between ubiquitin chains of different

linkage, others preferred Lys48-linked ubiquitin chains. One of the Rad23 (RADiation sensitivity abnormal 23) human homologues, HR23a, which as discussed below is a ubiquitin receptor associated with substrate targeting to the 26S proteasome, has a C-terminal UBA domain that binds with 3.6-fold higher affinity to Lys48-linked chains compared to Lys63-linked ones. This UBA domain, like many other UBDs, binds to monoubiquitin with significantly lower affinity, namely 70-fold lower than its binding to Lys48-linked ubiquitin chains. The structure of the HR23a C-terminal UBA domain–Lys48-linked diubiquitin complex revealed that the UBA sandwiches between the two ubiquitin moieties to form unique contacts (including some interactions with the diubiquitin linker region) and a significantly larger binding surface than it could form with a single monoubiquitin, which explains its preference for the Lys48-linkage (FIG. 5a).

Several UBDs can selectively bind to Lys63-linked ubiquitin chains. For example, NZF domains of TAB2 (TAK1 binding protein 2) or TRABID (TRAF binding domain) preferentially bind to Lys63- over Lys48-linked ubiquitin chains. The specificity determinants that promote NZF domain interaction with ubiquitin chains of certain linkage type are currently not known.

By contrast with other UBDs, DUBs must act at regions linking ubiquitin moieties into a chain in order to access the cleavage site between ubiquitin moieties. This family of proteins in particular exhibits a high degree of specificity for certain linkage types. For example, the DUBs IsoT and USP2 readily cleave Lys48- and Lys63-linked chains, but have significantly less activity towards linear chains. By contrast the DUB CYLD (cylindromatosis tumour suppressor), which is a negative regulator of NF-κB signalling, cleaves linear and Lys63-linked chains, but not Lys48-linked ones. Other examples of DUBs with explicit specificity exist for ovarian tumour (OTU) domains; the A20 OTU domain hydrolyses only Lys48-linked chains whereas the TRABID OTU preferentially binds and cleaves Lys63-linked chains.

Linker regions within a tandem repeat of UBDs can also define linkage specificity. For example, RAP80 (Receptor Associated Protein 80) targets BRCA1 to DNA damage-induced foci- through its two UIMs, which bind Lys63-linked ubiquitin chains, but not Lys48-linked ones. The sequence between the two RAP80 UIMs promotes an appropriate protein conformation such that the UIMs are positioned for efficient avid binding across a single Lys63 linkage, thus defining selectivity (FIG. 5b). By contrast, ataxin-3, a DUB linked to the development of spinocerebellar ataxia type 3, contains two UIMs with a two-residue linker sequence that determines Lys48-specific binding, . The specificity of tandem UIMs is interchangeable between Lys63- and Lys48-linkages by swapping the linker sequences of RAP80 and ataxin-3.

Thus, tandem UIMs can be spatially arranged in the context of full size proteins such that simultaneous, high-affinity interactions are favored with one ubiquitin chain linkage, but unfavorable or impossible with other linkages. Hence, the coordinated action of multiple UBDs can be used to sort substrates towards specific functional pathways according to their ubiquitin chain linkage type. These findings have provided, for example, a moleculer basis

to the observation that Lys63-linked chains has a role in DNA repair rather than proteasome targeting.

UBDs specific for linear ubiquitin chains

Linear ubiquitin chains in which ubiquitin monomers are conjugated through Gly to Met linkages (FIG. 3e) have been implicated in the activation of the NF-κB signalling pathways, . Several proteins that regulate this pathway, including NEMO (NF-κB essential modulator), ABINs (A20 binding inhibitor of NF-κB) and optineurin, contain the UBAN (ubiquitin binding in ABIN and NEMO) domain, which specifically binds to linear ubiquitin chains, , , . Binding of NEMO UBAN domain to monoubiquitin is undetectable *in vitro*, whereas binding to Lys63-linked diubiquitin is 100-fold weaker than to linear chains, . Specific mutations of NEMO that block interactions with linear ubiquitin chains impair the activation of the IKK (IκB kinase) complex and NF-κB in response to TNFα stimulation.

The UBAN domain of NEMO and linear diubiquitin form a heterotetrameric complex with two linear diubiquitin molecules on either side of the NEMO-UBAN coiled-coil dimer (FIG. 5c). Specificity for linear ubiquitin chains is provided by a continuous surface along the coiled-coil that interacts with the canonical Ile44-containing surface, the C-terminal tail of the distal ubiquitin and a novel interaction surface of the proximal ubiquitin. Two loops, one from the distal ubiquitin molecule (from Pro37 to Gln40) and the other from the proximal ubiquitin (from Glu92 to Glu94), interact with each other and with the C-terminal tail of the distal ubiquitin (from Arg72 to Gly76). These residues form a structural core, which holds the two moieties in a semi-fixed orientation, and provides the basis for specificity towards linear ubiquitin chains.

The specificity of UBDs for ubiquitin linkages can provide the *in vivo* significance for the activation of the NF-κB pathway. The NZF domain of TAB2 binds preferentially to Lys63 chains and is important for recruitment and activation of the serine threonine kinase TAK1 (TGF-beta activated kinase 1), which is required for activation of the NF-κB and MAPK (mitogen-activated protein kinase) pathways upon TNF stimulation. On the other hand, linear ubiquitin chains affect the IKK complex through direct binding to the UBAN domain in neighbouring NEMO, which in turn can activate the NF-κB pathway, .

It is likely that linear specific UBDs might be involved in regulation of additional cellular processes. For example, the UBAN containing protein ABIN-1 binds preferentially to linear chains and was suggested to act as a negative regulator of the NF-κB pathway. However, *ABIN1 -/-* mouse embryonic fibroblasts do not have any defect in TNFα-induced NF-κB activation. ABIN1-deficient mice die during the embryonic development due to massive apoptosis in the liver. It was suggested that ABIN1 functions as a ubiquitin-dependent anti-apoptotic sensor in mice.

Multivalent ubiquitin–UBD interactions

More evidence is emerging that multiplication of UBDs in an effector protein or associated complexes can provide a multivalent binding surface for long or complex ubiquitin chains.

Recent evidence indicates the functional importance of such arrangements to regulate endosomal sorting and DNA repair.

ESCRT-ing ubiquitylated cargoes

Endocytic sorting of ubiquitylated surface receptors, such as epidermal growth factor receptor (EGFR), for lysosomal degradation via multivesicular bodies (MVBs) is performed by a series of ubiquitin receptors incorporated into larger complexes named ESCRT, -. The machinery includes four complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III), which are required for sequential sorting of cargoes. Structural details of isolated components and their complexes for each of the ESCRT assemblies have been revealed in recent years, .

The cores of these complexes are structurally conserved, mostly with α-helical domains. The regions responsible for ubiquitin recognition, UIM in ESCRT-0, UEV (ubiquitin-conjugating enzyme E2 variant) in ESCRT-I, NZF and PH in ESCRT-II, are at the peripheries of the ESCRT complexes to enable efficient recruitment of ubiquitylated cargoes. As discussed below, there are substantial variations in the mode of ubiquitin recognition by these domains, which vary from yeast to mammalian systems. Recognition of multiple ubiquitin moieties attached to a trafficking cargo must be robust in order to control sorting of often bulky cargoes, but cannot be too strong for efficient and seamless trafficking, . The appropriate level of ubiquitin interaction with the ESCRT complexes is accomplished by the multivalency of the ubiquitin-recognizing subunits and by local concentration of ubiquitin decorated cargoes.

A remarkable example of multivalent interactions is the use of double-sided ubiquitin binding modes in which α-helical (single, double and triple) structures form two ubiquitin binding surfaces. HRS (hepatocyte growth factor-regulated tyrosine kinase substrate) is a part of the ESCRT-0 complex and its UIM, which forms a short α-helix, binds two ubiquitin molecules with equal affinity. The crystal structure of the UIM in complex with two ubiquitin molecules shows that both ubiquitin moieties use the canonical Ile44-containing patch, but interact with the UIM on opposing sides with pseudo two-fold symmetry (FIG. 5d). This binding mode is accomplished by a shift of two residues along the UIM sequence, which creates a very similar ubiquitin-binding surface on both sides of the helix.

There are other examples of such double-sided UIMs including human co-chaperone HSJ1 [because of human nomenclature](heat-shock protein DnaJ homolog) and kinase MEKK1 (MAP kinase kinase kinase 1), endocytic regulators EPS15 and EPS15R (epidermal growth factor receptor substrate 15 and EPS15 related, respectively). Interestingly Vps27, a yeast homologue of HRS, has two single-sided UIMs (Supplementary information S2a (figure)) whereas mouse EPS15 also has tandem UIMs, but only one of them is double-sided (Supplementary information S2b (figure)). It is conceivable that the double-sided UIM and/or tandem UIM arrangements evolved from copies of single-sided UIMs to provide additional levels of control.

An example of double-sided ubiquitin binding is also found in the ESCRT pathway. GGA (Golgi-localized, gamma-ear-containing) and TOM1 (Target of Myb1) function as ESCRT-0 complex associated proteins, . The GAT (GGA and TOM) domains of GGA3 and TOM1

fold into a three-helix bundle to form two ubiquitin binding surfaces, a high affinity one formed by its first and second helices (Supplementary information S2c (figure)), and a low affinity binding surface formed by its second and third helices, . Like in the case of UIMs, the dual ubiquitin recognition of these proteins might enhance their affinity towards multiply ubiquitylated surface receptors.

Multisite interactions in DNA damage pathways

A prominent example among the various DNA damage pathways regulated by ubiquitin signals is translesion DNA synthesis (TLS), which responds to damage-induced stalling at replication forks and provides a good example of the involvement of multiple UBDs in regulating transcriptional response to DNA damage. In the TLS pathway, DNA damage promotes monoubiquitylation on Lys164 of proliferating cell nuclear antigen (PCNA), a processivity factor that forms a sliding clamp around DNA. PCNA weakly interacts with numerous DNA polymerases, but monoubiquitylation of PCNA favours its preferential interaction with Y-family TLS polymerases, which possess UBDs (ubiquitin-binding motif (UBM) and UBZ). These error-prone polymerases are able to bypass the lesion, which would otherwise block DNA replication. Functional integrity of UBDs is essential for proper localization of TLS polymerases to replication foci and cellular survival following UV irradiation, . UBZ and UBM domains bind monoubiquitin with comparable affinities, but they interact via distinct surfaces: UBZ requires the Ile44-containing hydrophobic patch whereas UBM binds independently of Ile44, .

The presence of multiple functional UBMs in mammalian polymerases REV1 (reversionless 1) and pol iota led to the hypothesis that these UBMs interact with multiple ubiquitylated components of replication foci. In accordance, mutation of either of REV1's UBM domains partially reduces its localization to stalled replication foci induced by UV light. The effect is more severe in pol iota, as mutation of its UBM domains completely blocks its accumulation at replication foci during S phase, and this result is independent of DNA damage. The prominent role of the UBM domains does not seem to apply to all organisms, since UBM1 of Rev1 is not functional in yeast cells. Swapping experiments have revealed that specific UBDs in host proteins are linked to distinct functional roles, which cannot be assumed by other UBDs. For instance, an exchange of the UBZ domain of Werner helicase interacting protein 1 (WRNIP1), which is involved in regulation of genome stability, for pol iota's UBM1, both of which can bind ubiquitin, abolishes foci formation of WRNIP1, suggesting that different UBDs have distinct functional roles in the context of full size proteins.

Ubiquitin receptors at the proteasome

Although Lys48-linked ubiquitin chains are largely recognized as a major signal to target substrates for proteasomal degradation, new evidence indicates that an array of different ubiquitin chains can determine the kinetics and fidelity of substrate targeting to the proteasome. All of the observable ubiquitin linkages in HeLa cells, including Lys6, Lys11, Lys27, Lys33, Lys48 and Lys63, increase in abundance on inhibition of proteasomal degradation by the drug MG132. In yeast, an increase was observed upon proteasome inhibition for all ubiquitin linkages except Lys63, which was demonstrated to be the only

linkage that does not have a role in proteasomal degradation. This selection against Lys63 linkages for a role in proteasome targeting is possibly due to its preferential binding to non-proteasomal UBDs as discussed in previous sections, as Lys63-linked chains can signal for proteasomal degradation *in vitro*, and *in vivo*.

The only chain types that seem to be intrinsically impaired for stimulating substrate degradation are forked chains, in which a ubiquitin moiety is modified at two different Lys residues. Altogether, these data suggest that ubiquitin receptors at the proteasome do not select against chains of certain linkage type and that local factors determine the presentation of ubiquitin chains to the proteasome. The 26S proteasome contains a 20S core particle (CP), which performs substrate proteolysis and is capped at either or both ends by a 19S regulatory particle (RP). The RP prepares degradation substrates for passage through a narrow entry that leads to the catalytic centre of the CP. It contains ubiquitin receptors for substrate recognition as well as DUBs and ATPases to deconjugate ubiquitin chains and unfold degradation substrates, respectively.

Proteasomal ubiquitin receptors

In *Saccharomyces cerevisiae*, there are five known ubiquitin receptors that can support protein degradation by the proteasome; two of these, Rpn10 (S5a in humans) and Rpn13, are proteasome subunits, whereas the other three, Rad23, Dsk2 (dominant suppressor of KAR1), and Ddi1 (DNA-damage inducible 1), bind to the proteasome's RP reversibly. Orthologues of these proteins exist in higher eukaryotes, some of which also have multiple paralogues. In yeast, none of these proteins are essential and even strains with all five of these receptors deleted are viable, suggesting that other components can mediate substrate docking to the proteasome in their absence; some candidates have been implicated through crosslinking experiments, . In mice, RPN10 and RAD23 are essential, and might therefore have more specialized roles in higher eukaryotes.

RPN10 contains UIMs, which bind monoubiquitin and ubiquitin chains. The two UIMs of S5a adopt helical configurations and bind monoubiquitin independently due to their separation by flexible linker regions (Supplementary information S2d (figure)). In binding to Lys48-linked diubiquitin however the two UIMs bind simultaneously to the two ubiquitin moieties for a markedly increased affinity. S5a binds Lys48- and Lys63-linked chains equivalently, but exhibits significantly weaker affinity for a mixture of Lys29- and Lys6-linked chains. It also binds Lys11-linked ubiquitin chains, in accordance with their ability to effectively target substrates to the proteasome for degradation.

In contrast to S5a, RPN13 has only one ubiquitin interacting surface, which occupies the same structural domain used to dock this receptor into the proteasome, . As discussed above, this domain folds into a PH domain and three of its loops bind ubiquitin (FIG. 4e). With the exception of the shorter Rpn13 protein from *S. cerevisiae*, Rpn13 binds the DUB Uch37 and thereby recruits it to the proteasome's RP-. It seems to bridge substrate docking with ubiquitin chain deconjugation. Human RPN13 exhibits strong affinity for even monoubiquitin and is therefore expected to bind to chains of all linkage type. It does exhibit preference however for the proximal over the distal ubiquitin of Lys48-linked diubiquitin due to favorable contacts with the linker region. This preference is expected to render the

distal ubiquitin moiety available for other interactions, such as with Uch37. RPN13 binding to ubiquitin chains and UCH37 might simultaneously facilitate UCH37's distal end deubiquitylating activity- by orienting the ubiquitin moieties in a configuration favorable to hydrolysis. Future experiments are needed to test this model and whether RPN13's ubiquitin-binding capacity contributes to its activation of UCH37.

Shuttling ubiquitin receptors

Proteins containing ubiquitin-like (UBL) and UBA domains are able to recruit ubiquitylated substrates to the proteasome for degradation-. Their UBL domain can bind to a scaffolding protein in the base of proteasome's regulatory particle, Rpn1 (S2 in humans), as well as to Rpn13 and S5a-, while their UBA domains bind to ubiquitin, . Depending on their cellular protein levels UBL-UBA containing proteins can also inhibit the degradation of ubiquitylated substrates. Such inhibition has been hypothesised to occur because UBA domains sequester ubiquitin chains to in turn prevent deubiquitylation, which is prerequisite to substrate hydrolysis by the 20S core particle of the proteasome, , .

The different UBL-UBA family members exhibit different ubiquitin-binding preferences and modes. A human Rad23 orthologue, HR23a, effectively sequesters ubiquitin moieties of chains up through eight ubiquitin monomers and, as discussed above, its C-terminal UBA domain sandwiches between the ubiquitin moieties of Lys48-linked diubiquitin, making close contact with the linker region connecting the two moieties (FIG.3a). These properties explain HR23a's ability to inhibit substrate deubiquitylation and are markedly different from Rpn13's binding to and activation of the proteasomal DUB Uch37. They presumably make Rad23 proteins better shuttling factors for passing substrates to the proteasome, as substrates will more likely remain ubiquitylated during transport. There is evidence that certain proteasome substrates require specific ubiquitin receptors, . More studies are needed to define substrate selectivity requirements for specific receptors; however, specificity is expected to be particularly applicable in higher eukaryotes in which Rad23 proteins and S5a are essential for viability.

Ubiquitylation and ubiquitin binding

The functional relevance of the interplay between ubiquitylation and ubiquitin binding has been best demonstrated by the finding that multiple ubiquitin receptors in the endosome are monoubiquitylated and that this modification regulates their capacity to bind in trans to ubiquitylated cargo receptors, , -. This type of auto-modification, known as coupled monoubiquitylation, requires the presence of a functional UBD in the host protein to be auto-ubiquitylated-. Interestingly, many UBDs that bind to monoubiquitin can mediate such modification in their natural proteins and can also act as transferable elements to mediate monoubiquitylation of newly formed chimeric proteins.

Mechanistically, there seem to be different ways by which UBD-containing proteins can undergo coupled monoubiquitylation. It can be achieved through direct binding of the UBD to ubiquitin-coupled E2 enzymes, thus being E3-independent. Alternatively, an E3 ligase can bind to a UBD following its monoubiquitylation, as NEDD4 (Neural precursor cell expressed, developmentally down-regulated 4) does to EPS15's UIM, or a UBL domain

within the E3 ligase can bind to the UBD to promote its ubiquitylation. An example of the latter is provided by the prerequisite interaction between the UBL domain of RING-type E3 ligase Parkin and the UIM of EPS15 for Parkin-mediated ubiquitylation of Eps15. The coupled monoubiquitylation might lead to an intramolecular interaction between ubiquitin and the UBD, which could in turn prevent trans interactions with other ubiquitylated proteins, , . Loss of trans interactions can exert a negative-feedback control in regulatory networks that require transient and consecutive interactions between the UBDs and their functional targets. In addition the conformational change resulting from the intramolecular ubiquitin–UBD interaction might affect the functionality of other domains present in the protein, such as enzymatic activities or interactions with other cellular components.

Ubiquitylation of UBD-containing proteins might impact a variety of other functions, such as the assembly of larger protein complexes or protein oligomerization. For example, ubiquitylation of the adaptor protein NEMO could modulate its oligomerization status. Lys285 and Lys309 of NEMO are linearly ubiquitylated by the LUBAC ligase. The NEMO–linear diubiquitin crystal structure revealed that the non-covalent interaction between NEMO's UBAN motif and linear ubiquitin chains is possible when NEMO is ubiquitylated at Lys285 (trans interaction, Supplementary information S3 (figure 3)), but not when it is ubiquitylated at Lys309, which is at the heart of the ubiquitin-binding interface. Therefore, it is possible that that these two ubiquitylation events are used to regulate oligomerization of ubiquitylated NEMO, and in turn, the production of large agglomerates for more efficient activation of the IKK kinases within such complexes. Future experiments are required to test whether such regulated oligomerization occurs and has any possible role in IKK kinase activation.

Concluding remarks and future challenges

The role of ubiquitin as an important cellular signal has been well established in many biological processes, much like phosphorylation. This knowledge has been rapidly expanding with the discovery of close to two dozen UBDs with distinct capacities to bind ubiquitin modifications and regulate different cellular processes. Structural analyses of ubiquitin–UBD interactions have become an essential foundation for better understanding specificity in ubiquitin networks. Structures of distinct ubiquitin modifications in complexes with their respective UBDs have revealed dynamic changes both in ubiquitin signals and UBDs during their interactions. These complexes, although giving a large set of valuable data, have largely been composed of isolated UBDs and purified ubiquitin modifications (monoubiquitin or distinct ubiquitin chains) and thus lack an additional layer of information. This is particularly true for proteins that contain not only one UBD, but rather harbour several UBDs with different affinities and specificities for ubiquitin modifications (FIG. 4 and Supplementary information S2 (figure)). Structural determination of both full-length proteins and multimeric ubiquitin-mediated complexes remains one of the biggest challenges. At present, there is no single structure of any protein conjugated with ubiquitin, besides ubiquitin itself in structures of different ubiquitin chains.

The investigation of UBDs will surely expand and offer new surprises and opportunities. New classes of UBDs are continuously being reported, revealing novel information, not only

in regard to functionality but also to the mechanisms of intermolecular regulation. The finding that loops rather than secondary structural elements of a UBD can mediate ubiquitin binding indicates that distinct tertiary folds, which are not easily distinguishable as a modular domain, might create yet another set of ubiquitin-binding folds. Additionally, systems biology approaches are expected to expand our knowledge on the composition and behaviour of ubiquitin–UBD networks in various conditions. We have previously sought to define central hubs in ubiquitin–UBD networks and named them KRUBs (Key-nodes Regulated by UBiquitin). It will be important to expand the list of KRUBs and to describe their properties and dynamics using *in vivo* experimental approaches and *in silico* simulations. Finally, the accumulated knowledge of ubiquitin–UBD interactions will also impact pharmacological and medical applications (BOX 1). Several UBDs have now been linked to several human pathologies, including cancer and immune deficiencies, thus becoming interesting as putative targets for therapy.

Box1 | Targeting the ubiquitin–UBD interaction surface

Selective binding between ubiquitin signals and their specific ubiquitin-binding domains (UBDs) transmits intracellular signals controlling many biological functions, which are often altered in human pathologies. Ubiquitin–UBD interactions are characterized by low affinity and dynamic exchange, and often involve multiple surfaces within the protein complex. It is inherently difficult to use small chemical compounds to interfere with protein–protein interactions, especially those involving large binding surfaces. In the case of ubiquitin and UBDs, the interacting surface is generally flat and quite large. However, multiple mutagenesis studies indicate that their binding depends on a limited number of amino acid residues, which might represent ‘hot spots’ required for binding. Generally, good targets are surfaces that have hot spots that can be covered by a drug-sized molecule (see the figure, part **a**). A problem with targeting ubiquitin, especially its commonly used Ile44-containing hydrophobic patch, is that its disruption will affect numerous UBD interactions and signalling pathways, hence leading to side effects. Ubistatins were the first chemical compounds identified to interfere with ubiquitin–UBD interactions and these small molecules bind to ubiquitin chains and prevent their recognition by the 26S proteasome. Clinical use of ubistatins has been hampered due to poor bioavailability data; however, they provide an example that ubiquitin–UBD interactions can be efficiently disrupted by small compounds (see the figure, part **b**). Targeting specific UBD-containing effector proteins instead of ubiquitin is expected to allow the specific manipulation of distinct cellular processes. Progress in the structure based drug-design field exemplified by the development of small peptidomimetic inhibitors targeting proteins controlling apoptotic pathways in cancer cells, such as IAPs (inhibitors of apoptosis) and Bcl-2 (B-cell lymphoma 2) (reviewed in), indicates that it is pharmacologically possible to disrupt protein–protein interactions with small molecules. These approaches require novel chemical synthesis strategies for ‘peptidomimetic-like’ compounds; however, pharmacological development and usage of such modern therapeutics are promising.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biographies

Ivan Dikic received his M.D. and Ph.D. from the University of Zagreb, Croatia. After a postdoctoral tenure at New York University, he became a group leader at the Ludwig Institute for Cancer Research, Uppsala, Sweden. He has been Professor of Biochemistry, since 2002, and director of the Institute of Biochemistry, since 2009, at the Goethe University Medical School, Frankfurt. His group focuses on the role of ubiquitin (Ub) and Ub-like proteins as intracellular signals controlling receptor endocytosis, DNA repair and cancer pathogenesis.

Soichi Wakatsuki received his Ph.D. from Stanford University. After a postdoctoral study at Oxford University, he moved to the European Synchrotron Radiation Facility, Grenoble as a staff scientist and then a group leader until 2000. He is a Professor and Director of the Photon Factory, a national synchrotron radiation facility, and Structural Biology Research Center, KEK, Japan. His group uses synchrotron radiation X-rays to study ubiquitin pathways, protein glycosylation and vesicle transport.

Kylie Walters received her Ph.D. from Harvard University. After a postdoctoral tenure at Harvard Medical School, she became an Assistant Professor in Biochemistry, Molecular Biology, and Biophysics at the University of Minnesota, where she is now an Associate Professor. Her group uses NMR spectroscopy to study the ubiquitin-proteasome pathway.

Glossary terms

β-sheet

A secondary structure element of proteins in which the peptide backbone is almost fully extended.

3_{10} helix
A secondary structure element of proteins in which a coiled conformation enables hydrogen bonds between backbone carbonyl and amide groups of amino acids that are three residues apart in sequence.

α-helix
A secondary structure element of proteins in which a coiled conformation enables hydrogen bonds between backbone carbonyl and amide groups of amino acids that are four residues apart in sequence.

Residual dipolar coupling (RDC)
Dipole-dipole coupling between spin ½ nuclei in samples that are partially aligned with an external magnetic field. RDC provides the orientation of bonds between neighbouring atoms relative to the rest of the molecule and can therefore be used in structure determination by nuclear magnetic resonance spectroscopy.

Zinc finger
A small structural motif of proteins that is stabilized by interactions between amino acid sidechain atoms and a coordinated zinc atom.

Plekstrin homology (PH) fold
A protein structural fold characterized by a distinct pattern of β-strands and a α-helix. This protein family tends to have a core of bulky hydrophobic amino acids.

Ubiquitin-conjugating (UBC) domain
The α/β structural fold of E2 conjugating enzymes characterized by a conserved active-site Cys residue, which forms a thiolester bond with ubiquitin.

UBA domain
A small protein structural domain characterized by a three-helix bundle and typically associated by the ubiquitin pathway.

CUE domain
A protein structural domain akin to the UBA domain. CUE domains form a three-helix bundle.

Endoplasmic reticulum-associated degradation (ERAD)
A pathway by which misfolded proteins are transported from the endoplasmic reticulum to the 26S proteasome in the cytosol.

26S proteasome
A large protein complex that performs regulated degradation to control protein lifespan. Proteasome activity is essential for a large range of cellular events including cell cycle progression, DNA repair, apoptosis and the removal of misfolded proteins.

Coiled-coil
A structural motif in which α-helices coil around each other to enable favourable interactions between amino acid sidechain atoms.

Translesion synthesis polymerases
DNA polymerases that carry out translesion synthesis (TLS) past DNA lesions. These polymerases mostly belonging to the so-called Y-family have a more open configuration and can accommodate different damaged bases in their active sites.

UBAN domain
α-helical domain present in proteins NEMO, ABINs and optineurin. It binds specifically to linear ubiquitin chains.

Multivesicular bodies (MVB)
Specialised endosomes that are formed from early and sorting endosome by invagination of endosome membranes enriched with surface receptors resulting in many internalized vesicles. They mature into late endosome and lysosome for degradation of internalized surface receptors.

UBM domain
A structural ubiquitin binding motif present in translesion DNA polymerases and required for proper localization of these enzymes in nuclear replication foci.

UBZ domain
A subclass of ubiquitin-binding zinc finger domains able to bind to ubiquitin and controlling DNA damage responses.

Endosome
Small vesicles are formed by endocytosis (invagination) of plasma membranes along with surface receptors, and are responsible for sorting of internalized proteins and other biomolecules. Depending on the cargoes, endosomes are either transported back to the cell surface for recycling, transported to the Golgi apparatus, or matured into lysosomes.

Peptidomimetic inhibitor
Chemical or natural compound mimicking a peptide peptide interaction through a non-peptide bond or structure.

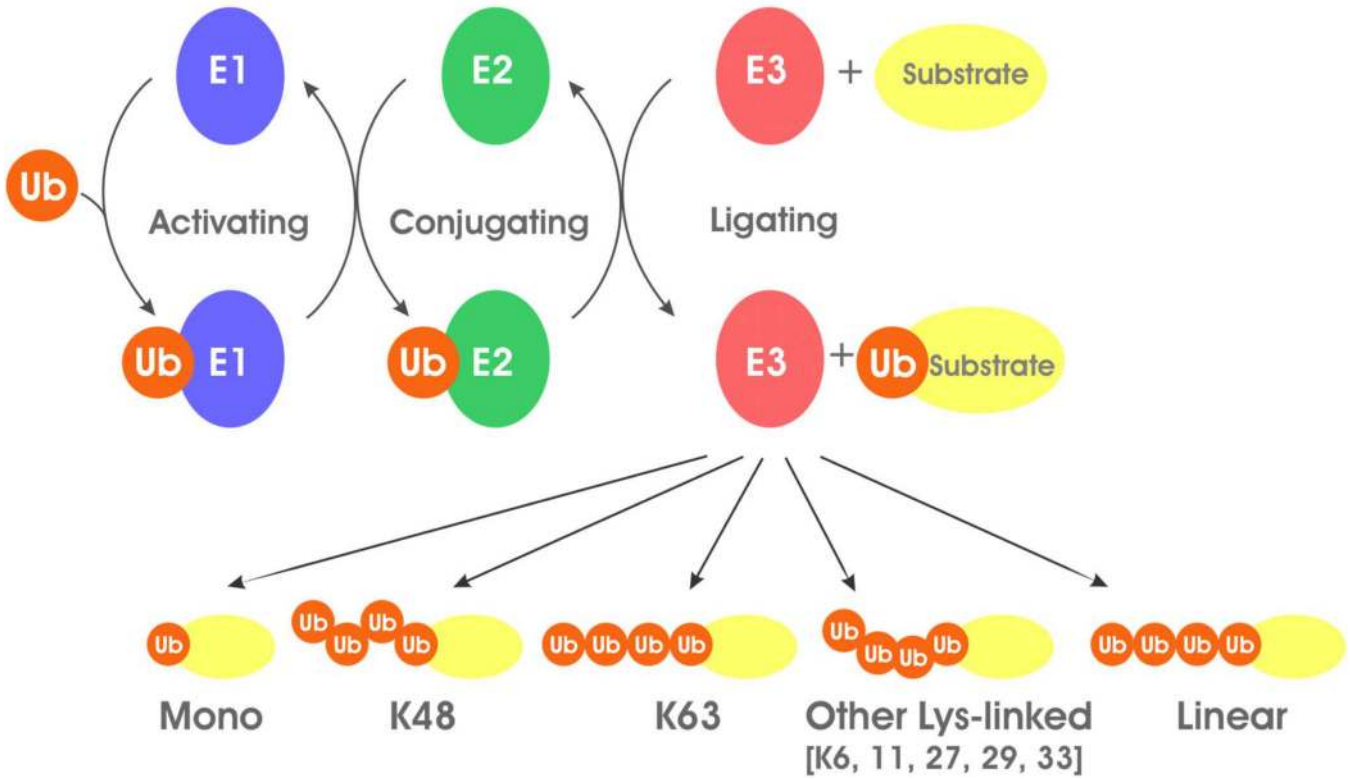

Figure 1. Enzymatic cascade that leads to substrate ubiquitylation

The activity of three enzymes is required for ubiquitylation, an E1 activating enzyme, an E2 conjugating enzyme and an E3 ligating enzyme, which recognizes the substrate. The completion of one cycle results in a monoubiquitylated substrate. However, the cycle can be repeated to form polyubiquitylated substrates. Additional ubiquitin molecules can be ligated to a Lys residue (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 or Lys63) in a previously attached ubiquitin to form Lys-linked chains. Alternatively, ubiquitin molecules can be linked head-to-tail to form linear chains. Only homotypic chains are shown, however, some E2-E3 combinations produce chains of mixed linkage.

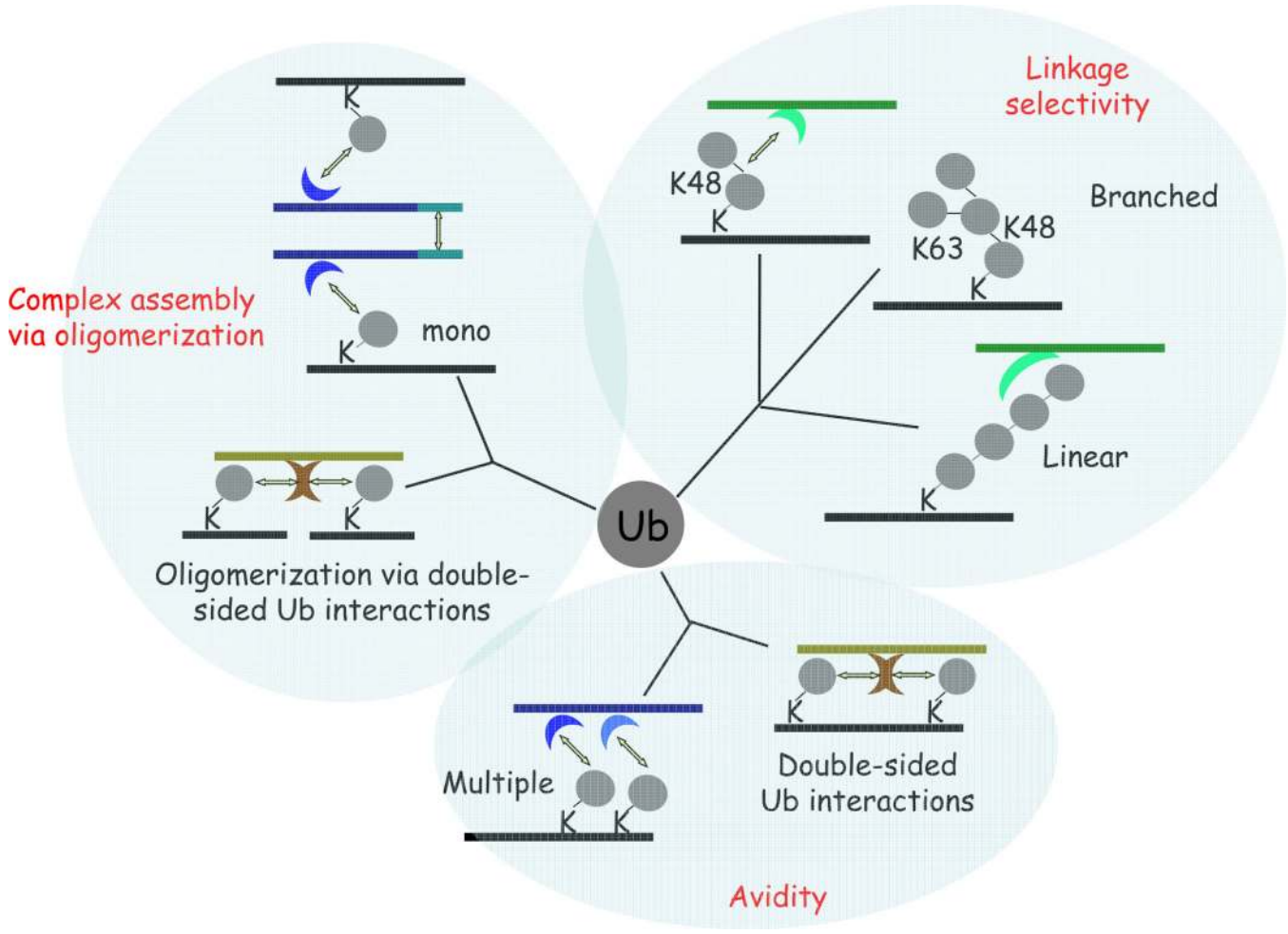

Figure 2. The ubiquitin–UBD network

A single type of molecule, ubiquitin, can be covalently attached to target proteins: as a single moiety (mono), as multiple single moieties (multiple), as chains coupled to the same lysine residue in ubiquitin (homotypic), mixed chains linked through different Lys residues in ubiquitin (branched) or head-to-tail bound ubiquitin moieties (linear). Specialized sets of ubiquitin-binding domains (UBDs) can read these post-translational modifications and mediate different outputs depending on the protein in which they are embedded. **a |** Two UBDs in the same protein can bridge two ubiquitylated targets. Alternatively, two proteins carrying oligomerization domains and UBDs can indirectly bridge the same ubiquitylated cargo. In all cases, this results in the formation of protein complexes, which might help to amplify a signal or activate a downstream activity. **b |** Specialized UBDs have also been discovered that are able to selectively discriminate between different types of ubiquitin chains. **c |** Lastly, the presence of two or more UBDs in a protein or the attachment of multiple ubiquitin moieties onto the same target can increase avidity and promote ubiquitin–UBD interactions despite their low affinity interactions. This phenomenon might be important to filter noise coming from non-specific transient ubiquitin–UBD interactions and to amplify only the output of proper ubiquitin–UBD pairs. UBDs that bind to one ubiquitin moiety are displayed as blue half moons, whereas those that interact specifically with the

regions linking ubiquitin moieties are in orange. Double-sided UBDs are displayed as brown half moons. Arrows indicate protein–protein interactions.

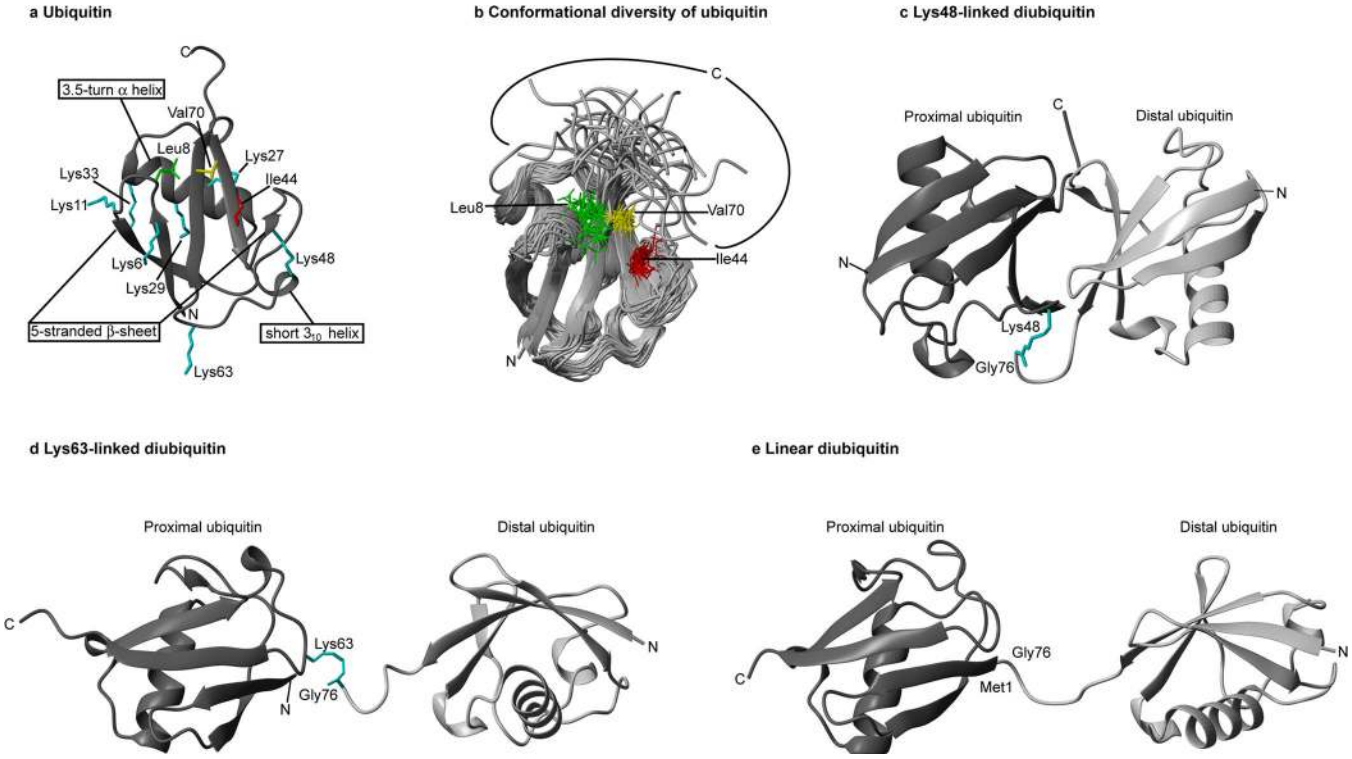

Figure 3. Structural diversity contributes to the multiplicity of ubiquitin signalling

a | Ubiquitin contains a 5-stranded β-sheet, a 3.5-turn α-helix and a short 3_{10} helix. Seven solvent-exposed Lys residues (displayed in blue) are available to assemble ubiquitin chains and the hydrophobic residues Leu8 (green), Ile44 (red) and Val70 (yellow) serve as a platform for many ubiquitin-binding domain (UBD) interactions. A ribbon representation of monoubiquitin is shown (PDB 1D3Z)**b** | Ubiquitin is a dynamic molecule in solution with conformational diversity. Distinct conformations are selected by individual UBDs. Several conformations adopted in solution are displayed to highlight ubiquitin's dynamic range of motions. Leu8, Ile44 and Val70 are shown in green, red and yellow, respectively. **c** | Ribbon representations of Lys48-linked diubiquitin (PDB 1AAR). **d** | Ribbon representations of Lys63-linked diubiquitin (PDB 2JF5). **e** | Ribbon representations of linear diubiquitin forming isopeptide bond between Met1 and Gly76 (PDB 2W9N) . Linkage of ubiquitin molecules into a polymer enhances structural diversity for robust signalling. The isopeptide bond linkage is shown in cyan in (c) and (d). Whereas Lys48-linked chains form compact structures due to inter-moiety interactions, Lys63-linked and linear ubiquitin chains are extended. In each case, the linker and its neighbouring region are chemically diverse.

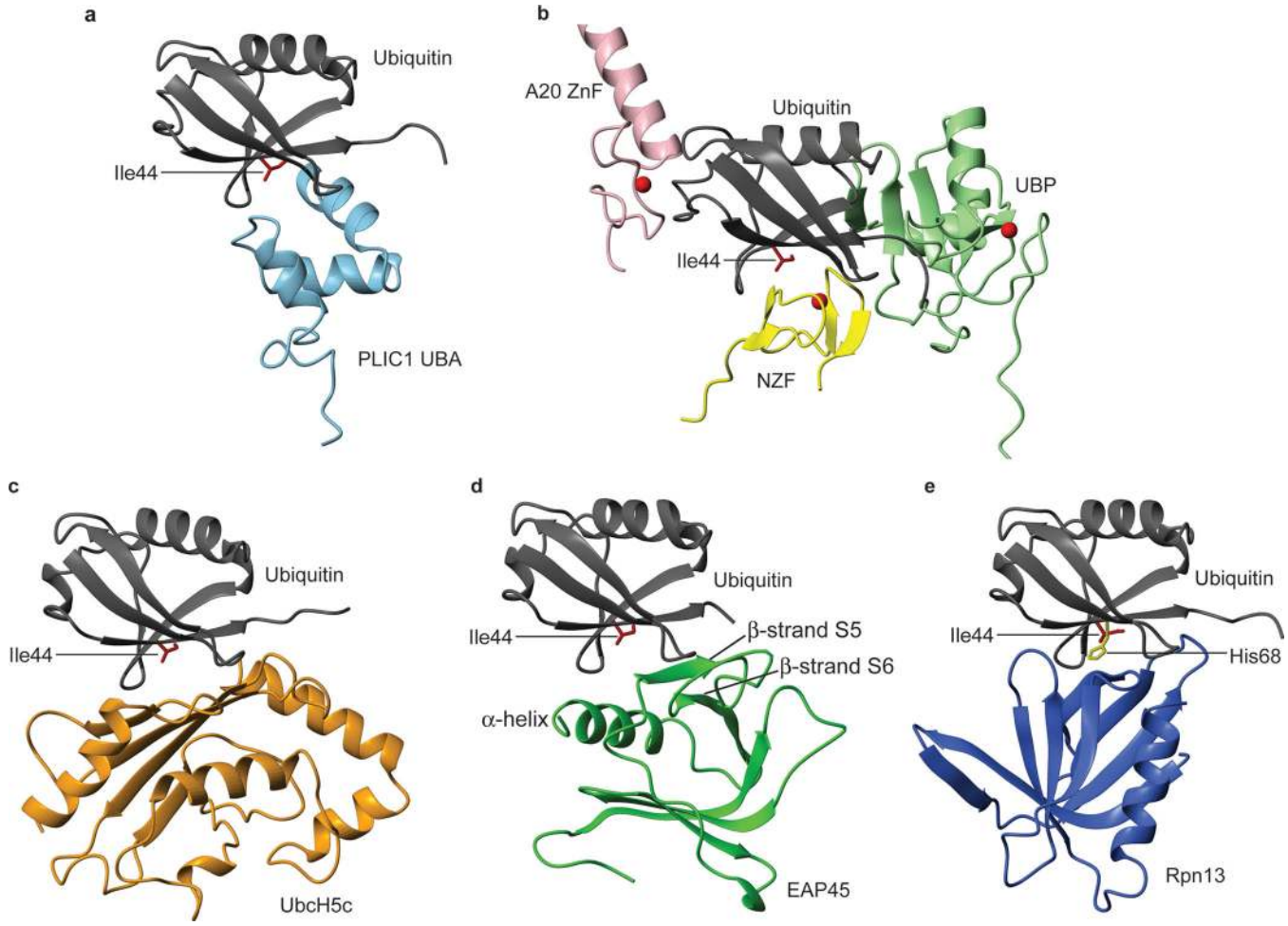

Figure 4. Ubiquitin is recognized by structurally diverse domains

Several ubiquitin– ubiquitin-binding domain (UBD) complexes are displayed with ubiquitin (grey) in the same orientation to highlight the common use of its β-strand surface to bind diverse UBD structures. **a** | Ribbon representations of PLIC1 (Protein linking IAP with cytoskeleton 1)] ubiquitin-associated (UBA) domain–ubiquitin (PDB 2JY6). **b** | Ribbon representations of ubiquitin-zinc finger domains of Rabex-5 A20 (PDB 2FIF), the NPL4 (nuclear protein localization 4) ZnF (NZF) domain (PDB 1Q5W) and isopeptidase T UBP (ubiquitin binding protein) (PDB 2G45) complexed with ubiquitin. **c** | Ribbon representations of ubiquitin in complex with the E2 ubiquitin-conjugating (Ubc) enzyme UBCH5c– (PDB 2FUH) **d** | GLUE (GRAM-like ubiquitin-binding in EAP45 domain– ubiquitin (PDB 2DX5;). **e** | Ribbon representations of RPN13 (Regulatory Particle, Non-ATPase-like 13)– ubiquitin (1-150; PDB 2Z59). The 3-helix bundle structure of the UBA domain (a), E2 ubiquitin conjugating domain (c), and plekstrin homology (PH) domain (d, e) all bind to ubiquitin's Ile44-centred hydrophobic patch, but do so in diverse manners. These UBDs cannot simultaneously act upon a common monoubiquitin or ubiquitin moiety within a chain. Zinc finger domains are more diverse in their binding to ubiquitin; Npl4 NZF binds to the Ile44-centred surface whereas Rabex-5 A20 ZnF and the deubiquitinase (DUB; also known as deubiquitylating or deubiquitinating enzyme) isopeptidase T UBP bind to an

Asp58-centred surface and the carboxyl terminus, respectively. In (b), the zinc atoms are displayed as red spheres, whereas in e), the sidechain atoms of His68 are displayed in yellow.

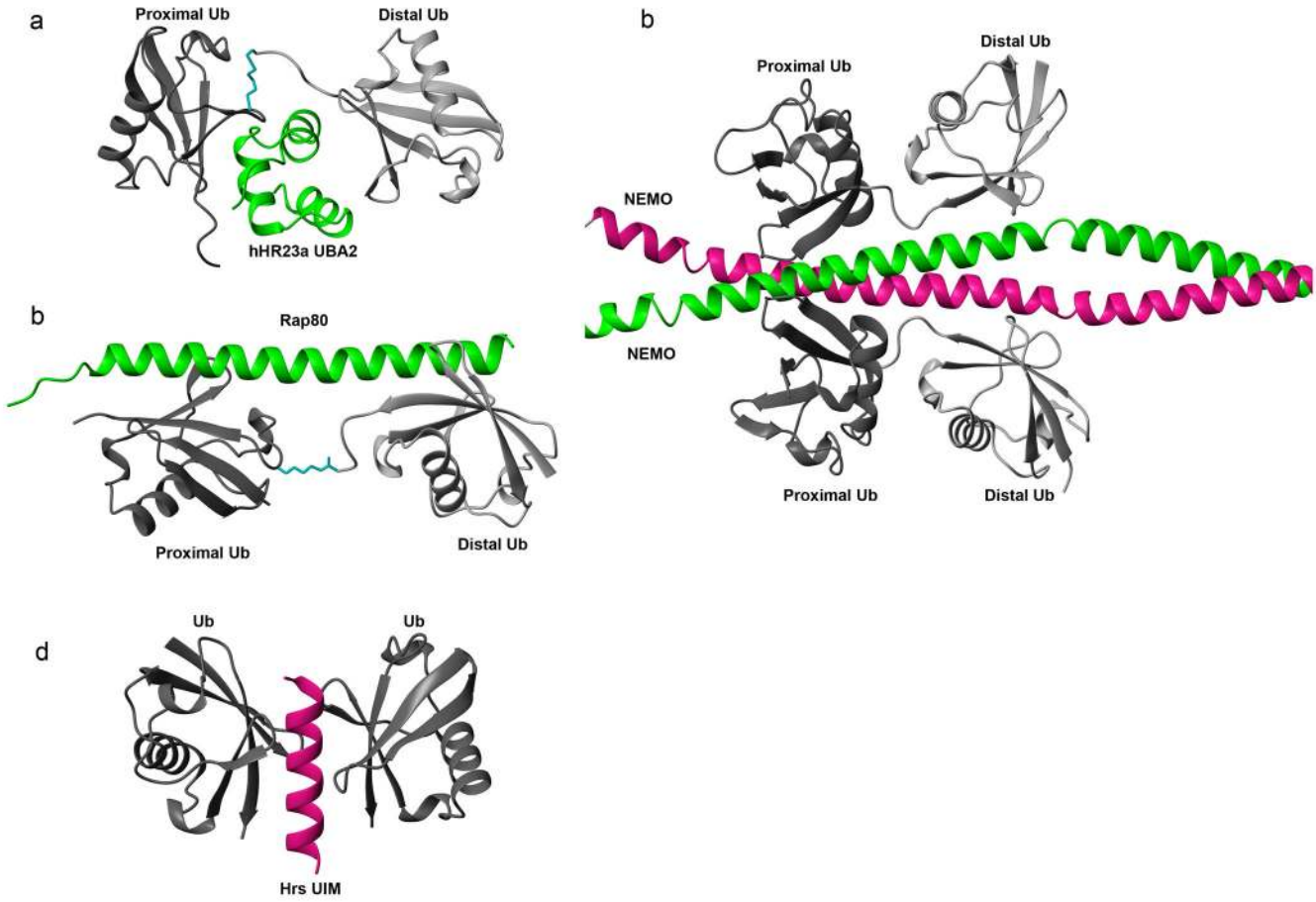

Figure 5. Multivalent interactions between ubiquitin and UBDs define chain specificity and increase affinity

a | HR23a ubiquitin-associated 2 (UBA2) domain (green) is sandwiched between the two ubiquitin moieties of Lys48-linked diubiquitin (grey) (PDB 1ZO6). This structure provides an explanation for this domain's preference for Lys48-linked chains, as the UBA domain contacts the ubiquitin linker region to significantly expand its binding surface beyond that possible for monoubiquitin. **b** | Structure of Lys63-linked diubiquitin bound to Rap80 (Receptor Associated Protein 80) illustrates why it binds to Lys63-, but not Lys48-linked chains (coordinates generously provided by Dr S. Fukai (University of Tokyo, Japan). Its contiguous helix binds simultaneously to two ubiquitin moieties, thereby increasing its affinity beyond that possible with monoubiquitin and defining the spacing between the two ubiquitin moieties to be greater than achievable with the Lys48 linkage. **c** | NF-κB essential modulator (NEMO) UBAN(ubiquitin binding in ABIN and NEMO) domain forms a coiled-coil, which binds two linear diubiquitins, . Extensive contacts are formed to both ubiquitin moieties thus conveying specificity for linear ubiquitin chains **d** | The structure of the single-helix double-sided HRS ubiquitin-interacting motif (UIM) bound to two ubiquitins reveals how it can target two ubiquitin molecules with equal affinity (PDB 2D3G). The ubiquitin molecules interact on opposite sides of this UIM and with a similar binding mode.

Table I
The functional and structural diversity of UBDs

Fold	UBD	Representative protein	Function	References
α helix	UIM	S5a (human) or Rpn10 (yeast), Vps27, STAM, EPSINs, RAP80	Proteasome degradation, endocytosis, MVB biogenesis, DNA repair	, ,
	MIU or IUIM	RABEX-5	Endocytosis	,
	DUIM	HRS	MVB biogenesis	
	UBM	POL-i, REV-1	DNA damage tolerance	
	UBAN	NEMO, ABIN1-3, OPTINEURIN	NF-κB signalling	, , ,
	UBA	Rad23/HR23A, Dsk2, NBR1	Proteasome targeting; kinase regulation; autophagy	, , ,
	GAT	GGA3, TOM1	MVB biogenesis	,
	CUE	Vps9, TAB2, TAB3	Endocytosis, kinase regulation	,
	VHS	STAM, GGA3	MVB biogenesis	
Zinc finger	UBZ	POL-h; POL-k, Tax1BP1,	DNA damage tolerance, NF-κB signalling	,
	NZF	NPL4, Vps36, TAB2, TAB3	ERAD, MVB biogenesis, kinase regulation	, ,
	ZnF A20	RABEX-5, A20	Endocytosis, kinase regulation	,
	Znf UBP (PAZ)	USP5/IsoT; HDAC6	Proteasome function, aggresome and autophagy [ok? We avoid using '/']	,
PH domain	PRU	RPN13	Proteasome function	,
	GLUE	EAP45	MVB biogenesis	,
Ubc-like	UEV	Uev1/Mms2	DNA repair, MVB biogenesis, kinase regulation	,
	Ubc	UBCH5C	Ubiquitin transfer	
Others	SH3	Sla1/CIN85	Endocytosis	
	PFU	Ufd3	ERAD	
	Jab1/MPN	Prp8	RNA splicing	

The number of ubiquitin binding domains (UBDs) is constantly growing, currently accounting for 20 different families, which are structurally classified into: α-helical, zinc fingers (ZnF), plekstrin homology (PH), the Ubc domain present in E2 enzymes and other folds. ABIN (A20-binding inhibitor of NF-kB signalling), CUE (coupling of ubiquitin conjugation to endoplasmic reticulum (ER) degradation), Dsk2 (dominant suppressor of KAR1), Ufd3 (ubiquitin fusion degradation 3), DUIM (double-sided UIM), EAP45 (ELL-associated protein of 45 kDa), ERAD (ER-associated degradation), GAT (GGA and TOM domain), GGA3 (Golgi-localizing, γ-adaptin ear homology domain, ARF-interacting proteins 3), GLUE (GRAM-like ubiquitin-binding in EAP45), HDAC6 (Histone deacetylase 6), HRS (Hepatocyte growth factor regulated tyrosine kinase substrate), JAB1 (Jun activation domain-binding protein 1)/MPN (Mpr1, Pad1 N-terminal), MIU (inverted ubiquitin-interacting motif (IUIM)), MVB (multivesicular body), NEMO (NF-kB essential modulator), NBR1 (Neighbor of BRCA1 gene 1), Npl4 NZF (nuclear protein localization 4) ZnF (NZF) domain, PAZ (Polyubiquitin Associated Zinc finger), PFU (PLAA family ubiquitin binding), Pol-I (DNA polymerase iota), PRP8 (Pre-mRNA-processing-splicing factor 8), PRU (PH receptor for ubiquitin), Rad23 (RADiation sensitivity abnormal 23)/HR23A, Rap80 (Receptor Associated Protein 80), REV-1 (Reversionless 1), Rpn10 (Regulatory Particle, Non-ATPase-like 13), SH3 (Src homology 3), Sla1 (Schizosaccharomyces pombe homolog of the human La protein), /CIN85 (Cbl interacting protein of 85 kDa), STAM (signal transducing adapter molecule), TAB2 (TAK1 binding protein 2), Tax1BP1 (human T-cell leukemia virus type I binding protein 1), UBA (ubiquitin-associated), UBAN (ubiquitin binding in ABIN and NEMO), UBC (ubiquitin-conjugating), UBP (ubiquitin binding protein), UBM (ubiquitin-binding motif), UBZ (ubiquitin-binding zinc finger), UEV (ubiquitin-conjugating enzyme E2 variant), USP5/IsoT (isopeptidase T), VHS (Vps27/Hrs/STAM), Vps27 (vacuolar protein sorting 27).