

UBIQUITIN-BINDING DOMAINS

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Abstract | Ubiquitin-binding domains (UBDs) are a collection of modular protein domains that non-covalently bind to ubiquitin. These recently discovered motifs interpret and transmit information conferred by protein ubiquitylation to control various cellular events. Detailed molecular structures are known for a number of UBDs, but to understand their mechanism of action, we also need to know how binding specificity is determined, how ubiquitin binding is regulated, and the function of UBDs in the context of full-length proteins. Such knowledge will be key to our understanding of how ubiquitin regulates cellular proteins and processes.

PROTEASOME

A large ~2.5-MDa multisubunit protein complex that binds to and subsequently degrades polyubiquitylated proteins.

POLYUBIQUITIN CHAIN

A string of ubiquitin molecules that are attached to each other by covalent bonds between the C terminus of one ubiquitin and any one of seven lysine residues of the adjacent ubiquitin (Lys48 and Lys63 are two of the key sites for polyubiquitin conjugation).

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Two proteins that need to bind to each other temporarily can be linked by interactions between a defined subdomain in one protein and a chemical group that is transiently attached to the other. A classic example of this process is provided by protein domains that bind to phosphorylated amino acids^{1,2}. This review examines interactions that result from ubiquitylation, a particularly remarkable form of post-translational modification in which an entire protein — **ubiquitin** — is covalently attached to free amino groups on lysine side chains or to the N-terminal amino group of a target protein^{3,4}. Ubiquitylation changes the molecular landscape of a protein, and can therefore influence the interactions of a protein with other proteins and, perhaps, the three-dimensional structure of a protein. We know that ubiquitin modification can alter protein location or activity to regulate many biological processes, including DNA repair, endocytosis, transcription and degradation or processing by the **PROTEASOME**^{5–10}. We also know something about the catalytic reactions (BOX 1) that select and append monoubiquitin or different **POLYUBIQUITIN CHAINS** to substrates (FIG. 1). However, in most cases, little is known about the biochemical mechanisms that are downstream of ubiquitylation events. Recent evidence indicates that these mechanisms involve the recognition of a specific ubiquitylated target by a 'downstream' ubiquitin-binding protein, which is also known as a **UBIQUITIN RECEPTOR**.

Ubiquitin-binding proteins generally have small (20–150 amino acid), independently folded ubiquitin-binding domains (UBDs) that can interact directly with monoubiquitin and/or polyubiquitin chains. UBDs can be found in enzymes that catalyse ubiquitylation or deubiquitylation (BOX 1), or in ubiquitin receptors that recognize and interpret signals from ubiquitin conjugated to substrate proteins. UBDs are structurally diverse and are found in proteins that contain different structural features and that have different biological functions (BOX 2). The presence of a UBD in a protein indicates that it can interact with ubiquitin or a ubiquitylated protein and might be regulated by ubiquitylation. Here, we introduce the known biochemical and biophysical features of UBDs, discuss mechanisms by which UBDs might be regulated and provide examples of how UBDs function.

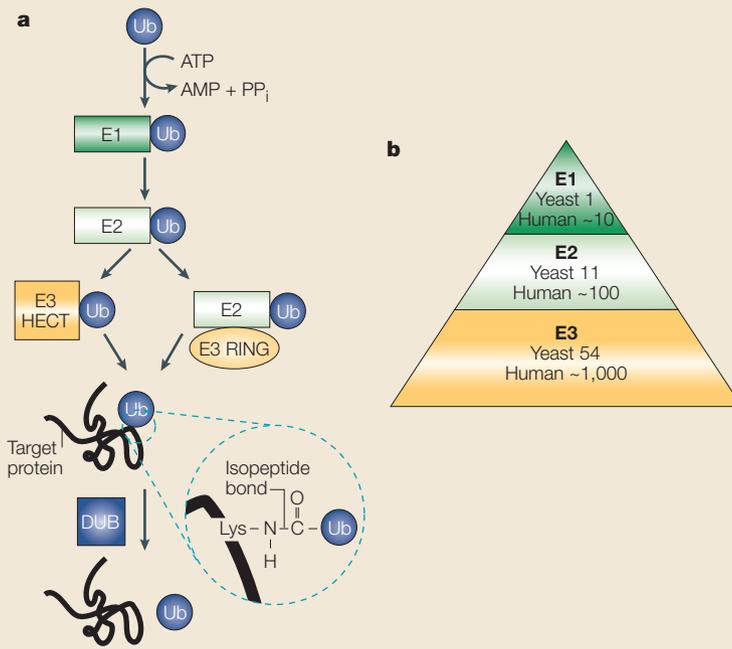
Characterization of ubiquitin-binding domains

As discussed below, and summarized in TABLE 1, nine modular UBDs have been identified.

Identification of UIMs and UBA domains. Polyubiquitin chains that are attached to a protein can provide a signal that targets the protein to the proteasome for permanent inactivation by degradation. Chains of ubiquitin that are attached to a condemned protein fulfil their role by binding either directly to a proteasome subunit or to a shuttling protein that

Box 1 | Ubiquitylation and deubiquitylation reactions

Ubiquitin is attached through its C terminus to exposed amino groups on proteins in a reaction that involves three enzymes — a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3) (REF. 4; see figure, part a). ATP is required to form a thiolester bond between the C-terminal carboxyl group of ubiquitin and the active site Cys of an E1. Further transfer of ubiquitin to a substrate requires two more enzymes, an E2 and an E3, which add two more layers of specificity to the reaction. Although there are only a few E1s present in each cell, there are many E2s and E3s (see figure, part b). The E1 passes ubiquitin to an E2, which is also linked to ubiquitin through a thiolester bond. E2s bind to specific E3 enzymes that bind to directly to the protein substrates. E3s can themselves form a thiolester bond with ubiquitin (HECT-domain E3s) before substrate attachment, or can function as a bridge between an activated E2 and its target (RING and U-box E3s). Ubiquitin is usually attached to the ϵ -amino group of a lysine through an isopeptide bond (see figure, part a), although ubiquitylation can also occur at the free N terminus of a protein. Ubiquitylation reactions are reversed by the action of deubiquitylating enzymes (DUBs) of which there are many types⁹⁵. PP_i, pyrophosphate; Ub, ubiquitin.



UBIQUITIN RECEPTOR

A ubiquitin-binding-domain-containing protein that is known to bind directly to ubiquitin and probably binds to ubiquitin signals in partner protein(s) rather than participating in ubiquitylation reactions.

S5A/RPN10

(subunit 5a (mammals)/regulatory particle non-ATPase-10 (yeast)).

A ubiquitin-interacting-motif-containing protein that is a stable subunit of the proteasome. It functions as one of the receptors for polyubiquitylated proteins that are destined for degradation.

HIDDEN MARKOV MODEL

A probability-based model that is used to make predictions about a set of uncharacterized sequences. The predictions are made on the basis of the conservation information that has been derived from a set of interrelated sequences.

YEAST TWO-HYBRID SCREEN

A method that is used to identify protein-protein interactions. It exploits the ability of two interacting proteins to bring the DNA-binding and DNA-activation fragments of a transcription factor together to trigger the expression of a reporter gene.

links ubiquitylated proteins to the proteasome. The first ubiquitin-binding site to be characterized was in a proteasome subunit, the S5A/RPN10 protein¹¹. The S5a sequence that is necessary and sufficient for interactions with ubiquitin is short and simple, and was used as a starting point in several bioinformatics searches to identify similar sequences in other proteins. HIDDEN MARKOV MODELS and iterative database searches that were based on the S5a sequence identified a sequence pattern known as the ubiquitin-interacting motif (UIM)^{12,13}. Like the original S5a UIM, UIMs in a number of diverse proteins were quickly shown to be direct, bona fide ubiquitin-binding motifs (for example, see REFS 14–17).

Another motif, the ubiquitin-associated (UBA) domain, was first identified using bioinformatics techniques as a sequence pattern that is common to a subset of proteins that are involved in ubiquitylation or deubiquitylation reactions¹⁸. At about the same time that UIMs were discovered, several laboratories showed that the UBA domain directly binds to ubiquitin^{19,20}. With the discovery of UIMs and UBA domains, the study of UBDs was launched. One particularly exciting observation was that UIMs and UBA domains are found in many proteins with diverse functions and locations (BOX 2), and are able to bind to both monoubiquitin and polyubiquitin chains. This diversity indicated that UIM- and UBA-domain-containing proteins might be receptors for many types of ubiquitin signal.

More ubiquitin-binding domains. On the heels of the UIM and UBA-domain discoveries, further ubiquitin-binding motifs were identified in YEAST TWO-HYBRID SCREENS. Screens using modified ubiquitin as bait to

identify monoubiquitin-binding proteins uncovered the CUE (coupling of ubiquitin conjugation to endoplasmic reticulum degradation) domain^{21,22}. The GAT (Gga and TOM1) and PAZ (polyubiquitin-associated ZINC FINGER) UBDs were found serendipitously when ubiquitin clones were isolated in two-hybrid screens that used bait proteins that were not previously known to bind ubiquitin^{23–26}. The PAZ domain was also discovered to bind ubiquitin in biochemical experiments²⁷, as was another type of zinc finger ubiquitin-binding motif, the NZF (Npl4 zinc finger) motif^{28,29}, as well as the VHS (Vps27, HRS, STAM)³⁰ and GLUE (GRAM-like ubiquitin-binding in Eap45)³¹ domains. At the end of the list is the UEV (ubiquitin-conjugating enzyme variant) motif, a domain that looks like the catalytic domain of E2s (ubiquitin-conjugating enzymes) but that lacks the active-site cysteine. Despite the structural relationship between the UEV and E2 catalytic domains, UEV domains are non-catalytic and function as non-covalent ubiquitin-binding sites in proteins with disparate functions.

Ubiquitin-binding characteristics of UBDs. Examples of each of the domains that are listed in TABLE 1 are known to bind directly to ubiquitin. For UBDs for which the dissociation constant (K_d) of the ubiquitin interaction has been measured, binding is moderate to weak, and there is a range of binding affinities within each class of domain (TABLE 1). In addition, several UBD families have members that do not seem to interact with ubiquitin at all^{15,28,32–35}, which emphasizes that binding domains that are identified by motif searches should be experimentally verified. Although one UBD that does not bind ubiquitin is thought to mediate homodimerization³⁴, the function

TOM1
(target of v-myb-1). A mammalian protein that contains both VHS (Vps27, HRS, STAM) and GAT (Gga and Tom1) domains and that probably functions in transport through the endosomal pathway.

ZINC FINGER
A protein domain in which the tertiary structure of the polypeptide chain is determined by coordination around a zinc ion.

NEDD8
(neuronal-precursor-cell-expressed developmentally downregulated protein-8). A protein with sequence (57% identity) and structural similarity to ubiquitin that modifies subunits of specific E3 ubiquitin-ligase complexes to increase their ubiquitylation activity.

UBIQUITIN-LIKE PROTEINS (Ubls). Motifs of 45–80 amino acids that have significant sequence and structural similarity to ubiquitin. Examples include discrete proteins and domains that are located within larger proteins.

ENDOCYTIC PATHWAY
The pathway that is followed by proteins that are internalized from the cell surface. Cargo proteins are either recycled back to the plasma membrane or move through early and late endosomal compartments to the lysosome.

EPS15
(epidermal growth factor receptor substrate-15). An important component of the endocytic machinery that functions in cargo sorting and vesicle formation at the plasma membrane.

EPS15-HOMOLOGY DOMAINS (EH domains). Domains of ~95 amino acids that mediate protein–protein interactions and are found predominantly, but not exclusively, in proteins that are involved in endocytosis. Many EH domains bind to motifs that contain an Asn-Pro-Phe (NPF) sequence.

of most UBDs that do not bind ubiquitin is unknown. Some UBDs might bind to NEDD8, which is a UBIQUITIN-LIKE PROTEIN (Ubl) that contains the conserved Ile44 patch (see below), or to more distantly related Ubls (for examples, see REFS 36–38).

Controversy surrounds the question of whether UBDs generally bind to monoubiquitin or polyubiquitin signals in the cell (FIG. 1a). Some members of each class of domain bind to monoubiquitin, so no single domain can be accurately referred to as a polyubiquitin-specific binding domain. On the other hand, binding assays indicate that most of the individual domains that can bind to monoubiquitin prefer to bind to polyubiquitin chains *in vitro* (for a possible exception, see REF. 28). Several domains even show a preference for Lys63-linked versus Lys48-linked chains^{29,39} (FIG. 1b). For some proteins, an *in vivo* preference for polyubiquitin chains makes sense, for example, for ubiquitin receptors that link polyubiquitylated proteins to the proteasome⁴⁰. For other UBD-containing proteins, such as those that are involved in membrane protein sorting in the ENDOCYTIC PATHWAY, the *in vivo* target is probably monoubiquitin, because endocytic cargo and the endocytic machinery are for the most part monoubiquitylated⁶. The type of ubiquitin partner that is preferred by an isolated UBD might differ from that preferred by the full-length protein or protein complex, or might be influenced by the subcellular location of the UBD-containing protein (for example, a protein that is tethered to the plasma membrane might only be exposed to monoubiquitylated substrates). The cellular, ubiquitylated partners of most ubiquitin-binding proteins are not known and will need to be identified before this discrepancy can be resolved. At this point, it seems that different members of a UBD class can recognize distinct types of ubiquitin modification in the cell. The individual domains of a class vary widely, not only in their ubiquitin-binding affinity and structural context, but perhaps also in the type of ubiquitin they are programmed to bind *in vivo*.

Many proteins carry multiple copies of a UBD. UIMs are often found in tandem, multiple UBA domains are present in several proteins (BOX 2), and several types of UBD can be found in the same protein or complex^{24,29,41} (see SMART in the Online links box). In one ubiquitin receptor, the endocytic protein EPS15, one UIM of a tandem arrangement of UIMs is unable to bind to ubiquitin^{15,32}. For two other endocytic ubiquitin receptors, biophysical data indicate that each UBD of tandem UIMs or CUE domains can bind ubiquitin completely independently^{32,42,43}. Some tandem domains have partially, but not completely, overlapping functions (for example, see REF. 17), which indicates that these domains do not bind to distinct ubiquitylated partners. Instead, each UBD might contribute to the affinity of the interaction with one partner type. In general, the reason why several UBDs are found in many proteins is not known and might vary from protein to protein.

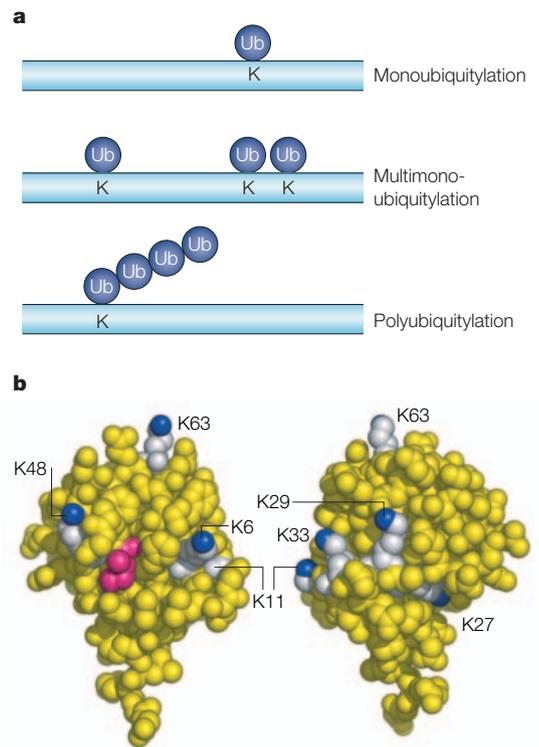


Figure 1 | Ubiquitin modifications. **a** | Monoubiquitylation is the modification of a protein with a single ubiquitin molecule on a single Lys residue. Multimono-ubiquitylation refers to the modification of several Lys residues with one ubiquitin. Polyubiquitylation is the modification of a protein, on one or more Lys residues, with a ubiquitin chain. **b** | Ubiquitin has seven Lys residues and all of them seem to be used for chain formation⁷² (the two opposite faces of ubiquitin are shown). Lys48- and Lys63-linked chains are the best characterized and are probably the most common. Because of the possible variations in the site and type of ubiquitylation, the possible configurations for ubiquitin modifications is vast. Lysines are depicted in white with the ϵ -amino-group nitrogen atoms in blue. All of the ubiquitin-binding domains that have been characterized so far contact an overlapping face on ubiquitin that includes Ile44 (red).

Why are UBD–ubiquitin interactions typically weak?
As discussed above, there is a wide range in UBD–ubiquitin affinities, but these interactions — especially those with monoubiquitin — are on the low-affinity end of the scale (they typically have a K_d of 10–500 μ M). Biologically relevant, low-affinity protein–protein interactions are not without precedent. For example, one cellular process that consists of many different types of low-affinity interaction is the assembly of a protein network that drives vesicle budding at the plasma membrane. Many of the proteins that are required for this process transiently associate with each other and with the membrane. Many types of interaction, such as those between EPS15-HOMOLOGY DOMAINS and Asn-Pro-Phe (NPF) motifs and between Src-homology-3 (SH3) DOMAINS and Pro-rich sequences, occur during network assembly, and these interactions have a K_d of 5–500 μ M (REFS 44–46). This clustering of high-specificity, low-affinity interactions creates a

Box 2 | The diversity of ubiquitin-binding-domain-containing proteins

Similar to Src-homology-2 (SH2) and Src-homology-3 (SH3) domains, ubiquitin-binding domains (UBDs) are found in proteins with disparate biological functions. UBDs also vary in the company they keep — that is, they are found in combination with various other domains and sequence motifs. To illustrate these observations, the proteins that carry one class of UBD — the ubiquitin-associated (UBA) domain — and that are encoded in the genome of the simple eukaryote *Saccharomyces cerevisiae* are shown in the figure. In this organism, there are 39 proteins that carry one or more of the UBDs that have been characterized so far. ArfGAP, ADP ribosylation factor GTPase-activating protein; DEAD, DExD/H-box helicase; EH, Eps15 homology; HA2, helicase associated; HELICc, helicase superfamily C terminus; RVP, retroviral aspartyl protease; RWD, RING finger/WD repeat/DEXDc-like helicase; STI1, heat shock chaperonin-binding motif found in the stress-inducible phosphoprotein STI1; UBL, ubiquitin like; ZnF-UBP, ubiquitin C-terminal hydrolase-like zinc finger.

| Yeast UBA protein | Human homologue | Domain structure | Function |
|-------------------|-----------------|--|---|
| Rad23 | HHR23A/B |  | Shuttling factor for proteasome degradation |
| Dsk2 | Ubiquilin-1/2 |  | Shuttling factor for proteasome degradation |
| Ede1 | EPS15 |  | Endocytic machinery that is required for cargo sorting and vesicle formation at the plasma membrane |
| Gts1 | ? |  | Nuclear protein that is involved in regulating metabolism |
| Ddi1 | HDD11/2? |  | Proteasomal degradation |
| Ubp14 | USP5, USP13 |  | Deubiquitylating enzyme, disassembles ubiquitin chains |
| Ylr419w | DHX29/36/57 |  | RNA helicase |
| Yor138c | None |  | Unknown |

SH3 DOMAINS

(Src-homology-3 domains). Protein domains of ~50 amino acids that bind primarily to short Pro-rich (PXXP) target sequences.

SH2 DOMAIN

(Src-homology-2 domain). Domains of ~100 amino acids that are found in diverse proteins. They can bind to a phosphorylated tyrosine residue of a partner protein.

ENVELOPED VIRUSES

Viruses that bud from infected cells and are surrounded by a lipid bilayer that is derived from the plasma membrane of the host cell.

TSG101

(tumour susceptibility gene-101). A UEV (ubiquitin-conjugating enzyme variant)-domain-containing protein that is required for the sorting of ubiquitylated cargo in the endocytic pathway.

GAG

A viral protein that carries a so-called 'late' domain that is required for the efficient budding of a virus from an infected cell. Low levels of ubiquitylated Gag have been detected in virus particles.

network that is fluid, can undergo rapid assembly and disassembly, and is only transiently stable following the formation of several modular-domain-peptide interactions. The system therefore has built-in 'dynamic instability'⁴⁷, which allows it to be readily reversible and enables assembly to proceed in a specific order of interactions. As a stable network requires numerous weak interactions, this situation also provides many targets for regulation because the disruption of any interaction could lead to network disassembly.

Weak UBD-ubiquitin interactions are probably physiologically relevant because point mutations in weakly-interacting UBD domains that inhibit ubiquitin binding are detrimental *in vivo* (for examples, see REFS 17,33,48). UBD-ubiquitin interactions might be relatively weak because they function in readily reversible, transitory protein networks similar to the one described above (UBD-ubiquitin interactions are probably part of the network required for the budding of plasma membrane vesicles, because numerous endocytic proteins have UBDs and/or are monoubiquitylated⁶). In these cases, the modification of a protein with ubiquitin would function as a switch that is recognized by UBDs and that controls the regulated assembly of a network, as has been proposed

for Src-homology-2 (SH2)-DOMAIN-phosphotyrosine interactions and other regulatory switches⁴⁹. Thanks to the presence of many deubiquitylating enzymes (DUBs) in most cells, ubiquitin-induced switches can be readily reversed and individually regulated.

Another reason for low-affinity UBD-ubiquitin interactions might be the relatively high concentration of free ubiquitin in cells (estimated to be ~10 μ M in mammalian cells⁵⁰). An exposed UBD with high affinity would be constitutively occupied with free ubiquitin and unavailable for binding to a ubiquitylated partner. So, in cases for which a higher affinity interaction between a low-affinity ubiquitin receptor and a ubiquitylated partner is required, the interaction could be substantially enhanced by the presence of several UBD motifs in the receptor or receptor complex, by the multimerization of ubiquitin receptors, or by further contacts between the ubiquitin receptor and the ubiquitylated target. ENVELOPED VIRUSES might use the last strategy to enhance the interaction between a host protein and a ubiquitylated viral protein to promote virus budding. Tumour susceptibility gene-101 (TSG101) is a cellular protein that has a low-affinity UEV domain (K_d ~500 μ M)⁵¹. TSG101 binds to the viral protein GAG and ubiquitin individually, but has a higher affinity

Table 1 | Ubiquitin-binding domains that have been characterized so far

| Domain* | Number of proteins in proteome [†] | Length (amino acids) | Method of identification | Ubiquitin-binding affinity (K_d) [§] | Selected references |
|--|---|----------------------|---|--|---------------------------------|
| CUE | <i>S. cerevisiae</i> 7 <i>H. sapiens</i> 21 | 42–43 | Two-hybrid analysis with monoubiquitin bait | ~2–160 μ M (monoubiquitin) | 21,22,42,57,70,97 |
| GAT | <i>S. cerevisiae</i> 2 <i>H. sapiens</i> 14 | 135 | Two-hybrid analysis with GGA1 clathrin adaptor bait; binding to monoubiquitin | ~180 μ M (monoubiquitin) | 25,26,41,85,96,98 |
| GLUE | <i>S. cerevisiae</i> ? <i>H. sapiens</i> ? | ~135 | Binding to monoubiquitin | ~460 μ M (monoubiquitin) | 31 |
| NZF (subset of ZnF-RBZ with a Thr-Phe motif) | <i>S. cerevisiae</i> 1 <i>H. sapiens</i> 25 | ~35 | Binding to monoubiquitin, polyubiquitin chains and ubiquitylated proteins | ~100–400 μ M (monoubiquitin) | 28,29,33,99 |
| PAZ (ZnF-UBP) | <i>S. cerevisiae</i> 5 <i>H. sapiens</i> 16 | ~58 | Two-hybrid analysis; binding to immobilized GST-ubiquitin | Not known | 23,27 |
| UBA | <i>S. cerevisiae</i> 10 <i>H. sapiens</i> 98 | 45–55 | Two-hybrid screens; binding to ubiquitin-sepharose | ~10–500 μ M (monoubiquitin) ~0.03–9 μ M (polyubiquitin) | 18–20,28,38,53,56,58,81,100–102 |
| UEV (subset of UBCC without the catalytic Cys residue) | <i>S. cerevisiae</i> 2 <i>H. sapiens</i> ? | ~145 | Similar to E2 catalytic domain; binding to immobilized GST-ubiquitin | ~100–500 μ M (monoubiquitin) | 48,51,59,61,62 |
| UIM | <i>S. cerevisiae</i> 8 <i>H. sapiens</i> 71 | ~20 | Bioinformatics based on the ubiquitin-binding site of S5a | ~100–400 μ M (mono- or polyubiquitin) | 12,14–17,32,35,43,103 |
| VHS | <i>S. cerevisiae</i> 4 <i>H. sapiens</i> 28 | 150 | Binding to monoubiquitin and polyubiquitin chains | Not known | 24,30 |

*Standard ubiquitin-binding-domain names that are not used in the SMART or Pfam databases (see the Online links box) are followed in parentheses by a SMART/Pfam designation and, if needed, further identifying features. [†]The data in this column come from the SMART or Pfam databases (see the Online links box) or from the published literature. [§]For comparison, K_d values are typically 1–10 μ M for Src-homology-3 (SH3)-domain-Pro-rich peptide interactions and 0.1–1 μ M for SH2-phosphotyrosine interactions¹. ^{||}Two independent sites in the three-helix bundle at the C terminus of the GAT domain bind to ubiquitin⁹⁷. CUE, coupling of ubiquitin conjugation to endoplasmic reticulum degradation; E2, ubiquitin-conjugating enzyme; GAT, Gga and Tom1; GGA1, Golgi-localized, γ -ear-containing, ADP-ribosylation-factor-binding protein-1; GLUE, GRAM-like ubiquitin-binding in Eap45; GST, glutathione-S-transferase; *H. sapiens*, *Homo sapiens*; *S. cerevisiae*, *Saccharomyces cerevisiae*; UBA, ubiquitin-associated; UBCC, ubiquitin-conjugating enzyme catalytic domain; UEV, ubiquitin-conjugating enzyme variant; UIM, ubiquitin-interacting motif; VHS, 'Vps27, HRS, STAM'; ZnF-RBZ, zinc finger domain in Ran-binding and other proteins; ZnF-UBP, ubiquitin C-terminal hydrolase-like zinc finger.

for ubiquitylated Gag⁵¹. In another example, although ubiquitin-binding regions in the catalytic domains of ubiquitin-pathway enzymes are not generally counted as UBDs, a DUB has been shown to bind simultaneously to conjugated ubiquitin and to a region of the ubiquitylated protein through interactions that are individually weak, but that together enforce specificity⁵². Similar to these examples, other UBD-containing proteins might increase their specificity and affinity by making contacts with a ubiquitylated target using protein regions that are outside of their UBDs.

Finally, a stable UBD-ubiquitin interaction might occur in cases in which a ubiquitin receptor is itself ubiquitylated (see below) and there is an intramolecular interaction between the conjugated ubiquitin and the UBD. In this case, because of the high local ubiquitin concentration, the UBD-ubiquitin affinity does not need to be high.

Regulation of ubiquitin-binding domains

One way to regulate the occupation of UBDs is to control their accessibility. Several UBDs bind to ubiquitin more effectively when they are outside the context of the full-length protein (for example, see REFS 22,27), which indicates that interactions between ubiquitin and UBDs are controlled by inter- or

intramolecular interactions, or by post-translational modifications. By contrast, the UBA domain in the full-length p47 protein binds to ubiquitin more effectively when p47 is associated with one of its partners^{28,53}, which indicates that the exposure of this UBA domain is regulated by tertiary or quaternary structural changes.

UBD accessibility might be controlled by steric occlusion. In one ubiquitin receptor that shuttles proteins to the proteasome, RAD23, ubiquitin binding is inhibited by an intramolecular interaction between a UBA domain and a Ubl domain^{36,38}. In another example, the binding of ubiquitin to the TOM1 GAT domain competes with binding of another GAT domain partner⁴¹. There are further examples of UBDs that interact with proteins other than ubiquitin^{54,55}, and whether UBDs generally have several partners is an open question. However, it is clear that participation in other intra- or intermolecular protein-protein interactions is a mechanism that regulates the ubiquitin-binding ability of some UBDs. It is probable that other mechanisms of UBD regulation remain to be discovered, with probable candidates including those that involve post-translational modification and the control of subcellular localization.

p47

A protein that binds to Cdc48 (also known as p97/VCP) to promote membrane fusion during the reassembly of the nuclear envelope, endoplasmic reticulum and Golgi complex after mitosis. The ubiquitin-associated (UBA) domain of p47 is required for p47-Cdc48 fusion activity.

RAD23

A protein that has ubiquitin-like and ubiquitin-associated domains and that functions as a receptor to shuttle ubiquitylated proteins to the proteasome.

Structures of ubiquitin-binding domains

The high level of sequence and structure conservation for ubiquitin throughout evolution presumably results from the fact that it needs to be able to bind to many different, structurally diverse UBDs. Various UBD–ubiquitin complex structures that have been determined using X-ray crystallography or NMR spectroscopy are shown in FIG. 2 (for the relevant references, see FIG. 2). These structures are strikingly varied: UIMs form a single α -helix that interacts with ubiquitin; NZF motifs bind to ubiquitin primarily through three residues, which are located on loops that emanate from four strands that are ordered by a zinc ion; CUE, UBA and GAT domains form three-helix bundles and two of these α -helices pack against ubiquitin; and UEV domains comprise a mix of β -strands and α -helices, and contact ubiquitin through two loops and part of the β -sheet.

A UBA-domain–ubiquitin complex structure⁵⁶ superimposes on the CUE-domain–ubiquitin structures^{42,57} (FIG. 2), which indicates that the CUE and UBA complex structures are closely related. By contrast, theoretical studies have indicated that two UBA domains of a different protein contact ubiquitin through the same surface, but use orientations that are different to each other and to that for CUE domains⁵⁸. It will be important to validate these theoretical studies experimentally.

Like the CUE/UBA complexes, GAT domains pack two α -helices against ubiquitin (FIG. 2). However, the order in which the helices are connected to each other is different for CUE/UBA and GAT domains, and the two ubiquitin-binding helices of CUE/UBA domains are antiparallel, whereas the GAT helices are parallel. The resemblance between the CUE/UBA and GAT domains and the single helix of UIMs is also superficial (FIG. 2), because the UIM helix and the two helices of CUE/UBA or GAT domains lie across ubiquitin at angles that differ by $\sim 60^\circ$. So, a number of completely different folds use diverse surfaces — single helices, two adjacent helices, β -strands or loops — to bind ubiquitin.

Another unexpected twist is found on comparing the two available CUE-domain–ubiquitin complex structures. In one case, ubiquitin binds to a single CUE domain⁴², whereas in the other, ubiquitin binds to a CUE dimer. In the dimeric structure, one of the α -helices in each domain is swapped, which forms a structure that is kinked to allow the two equivalent CUE-domain surfaces to contact different portions of the same ubiquitin molecule⁵⁷ (FIG. 2). This structural contortion expands the CUE-domain–ubiquitin interface from 550 \AA^2 to 870 \AA^2 , such that it includes residues near the C terminus of ubiquitin. This type of binding interaction probably explains the relatively high binding affinity of this particular CUE-domain-containing protein for ubiquitin⁵⁷.

Further complexity is provided by UEV-domain-containing proteins. The UEV domains of human TSG101 (REF. 59) and its *Saccharomyces cerevisiae* orthologue, Vps23 (vacuolar protein sorting-23; REF. 60), bind to ubiquitin through a distinctive

' β -tongue' structure (FIG. 2) that is not found in other UEV-domain-containing proteins. By contrast, a UEV domain that is found in the E2 subunit MMS2 — the ubiquitin-binding surface of which has been inferred from mutagenesis and NMR studies^{61,62} — uses a different ubiquitin-binding interface. Remarkably, the two ubiquitin-binding interfaces that have been characterized for TSG101/Vps23 and Mms2 both differ from the contact surface that has been characterized between an E2 enzyme and its thioester-linked ubiquitin^{62–64}. So, the related UEV/E2 structures bind to ubiquitin through three completely distinct interfaces. These examples illustrate the remarkable adaptability of protein architectures and show that the ubiquitin-binding function can be built into numerous folds, a capability that is probably facilitated by the low binding affinity of most of the ubiquitin interactions that have been characterized so far.

Interaction surfaces on ubiquitin

Although there are no obvious common themes for UBD structures or for the UBD surfaces that contact ubiquitin, the same cannot be said for the ubiquitin side of the partnership. All of the UBDs that have been characterized so far contact an overlapping face on ubiquitin that includes Ile44 (FIG. 2). This is facilitated, in part, by the ability of the residues that surround Leu8 — and to a lesser extent, Gly47 — to move on either side of the interface. Although the interfaces overlap, they are far from identical; the various UBD footprints on ubiquitin show substantial variation, and there is little or no equivalence in specific residue interactions.

Overlapping binding surfaces could be important in pathways in which ubiquitylated cargo might be passed along a series of proteins by sequential interactions with different UBDs (for instance in the late endocytic pathway; see below). Because the same ubiquitin moiety cannot interact simultaneously with two domains that share overlapping ubiquitin-binding interfaces, ubiquitin 'hand-off' from a ubiquitin receptor would require further interactions, perhaps with ubiquitin or with a second ubiquitin receptor (for example, see REF. 65). Differences in UBD footprints on ubiquitin have other functional implications. For example, some UBDs approach Lys48 or Lys63, which are two of the key sites for polyubiquitin conjugation. This might be of relevance to the recognition of polyubiquitin chains versus monoubiquitin^{20,38,42}.

In addition to the UBDs, ubiquitin also makes extensive interactions with the numerous and varied ubiquitin-activating (E1), E2, ubiquitin ligase (E3) and DUB enzymes of the ubiquitin pathway (BOX 1). Remarkably, all of the interactions between ubiquitin and these enzymes that have been characterized so far also use the Ile44 face of ubiquitin^{52,64,66,67}. The large number of different interactions that occur between ubiquitin and UBDs or ubiquitin-pathway enzymes probably explains why the Ile44 face of ubiquitin has been highly conserved throughout evolution. However, it does not explain why the opposite face is also conserved: 13 of the surface residues of ubiquitin

MMS2
A UEV (ubiquitin-conjugating enzyme variant)-containing protein that binds to Ubc13 (ubiquitin-conjugating enzyme-13) to form an E2 complex that functions specifically in the formation of Lys63-linked polyubiquitin chains.

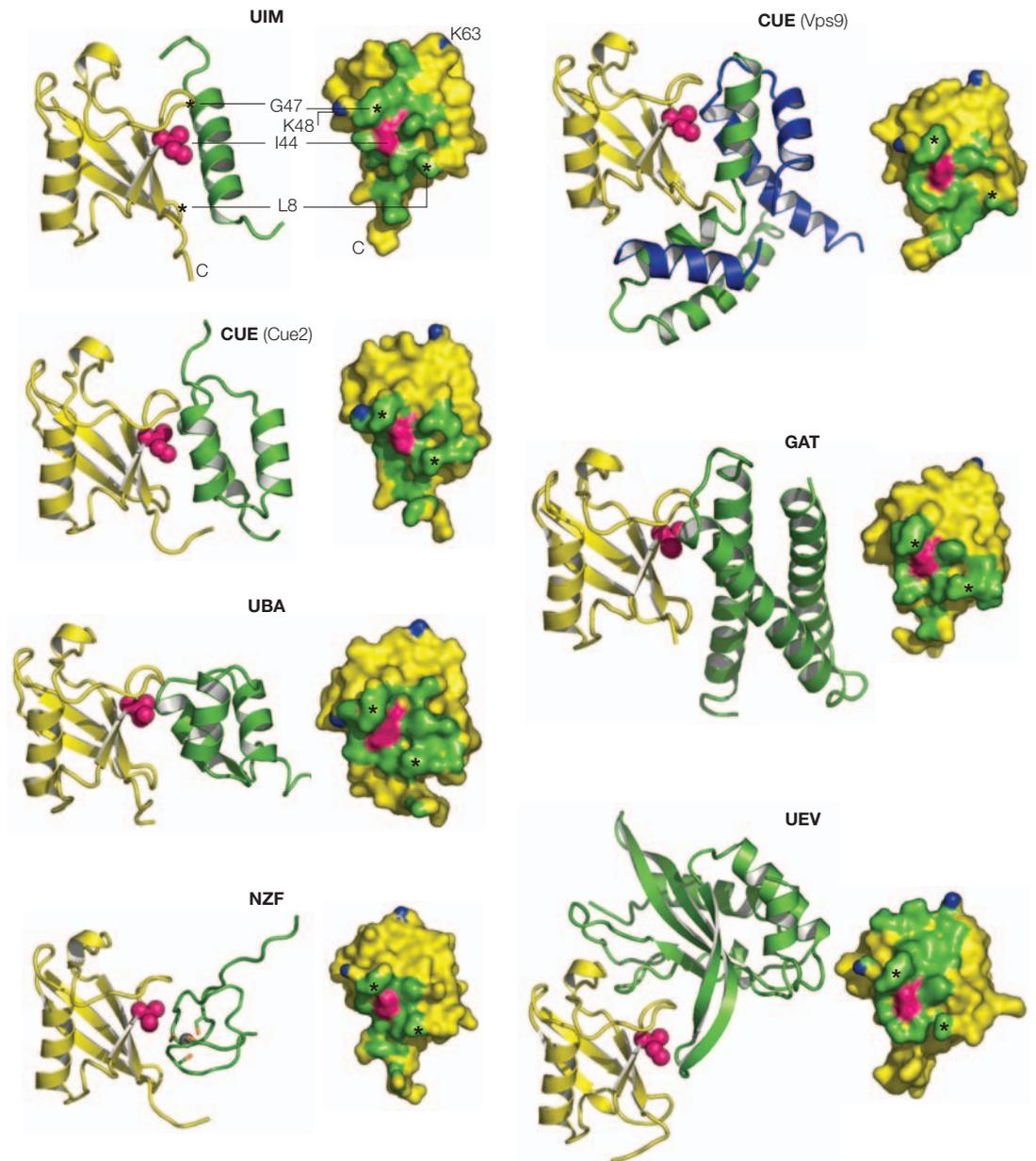


Figure 2 | **Ubiquitin–ubiquitin-binding-domain complex structures.** Shown are ubiquitin–ubiquitin-binding-domain (UBD) complex structures that were determined using X-ray crystallography or NMR spectroscopy. For each UBD, the left panels show ribbon diagrams in which ubiquitin (yellow) is always shown in the same orientation, and the UBDs are coloured green (green and blue for the CUE dimer). Ile44 of ubiquitin is shown in a red, spacefill representation. The right panels show ubiquitin with the UBD contact surfaces coloured green, although the Ile44 side chain is again shown in red and is always part of the contact surface. The C terminus of ubiquitin adopts a wide range of different conformations. The amino groups of Lys48 and Lys63, which are two of the key sites for polyubiquitin chain conjugation, are coloured blue. Leu8 and Gly47, which move to accommodate the different UBD partners, are highlighted by asterisks. The structures that were used to construct this figure are: UIM (ubiquitin-interacting motif; Protein Data Bank (PDB) accession code 1Q0W)⁴³; CUE (coupling of ubiquitin conjugation to endoplasmic reticulum degradation) domain from Cue2 (PDB accession code 1OTR)⁴²; UBA domain (ubiquitin-associated domain; PDB accession code 1WR1)⁵⁶; NZF (Npl4 zinc finger; PDB accession code 1Q5W)³³; CUE domain from vacuolar protein sorting-9 (Vps9; PDB accession code 1P3Q)⁵⁷; human GGA3 (Golgi-localized, γ -ear-containing, ADP-ribosylation-factor-binding protein-3) GAT (Gga and Tom1) domain (PDB accession code 1YDB)⁹⁶; and UEV (ubiquitin-conjugating enzyme variant; PDB accession code 1S1Q)^{59,60}.

do not participate in any of the characterized interactions with enzymes or UBDs, yet all but three of these residues are invariant between humans and *S. cerevisiae*. The obvious inference is that other functionally important interactions of ubiquitin remain to be discovered.

Coupled ubiquitin binding and ubiquitylation
Curiously, almost as soon as UBDs were discovered, it was established that many ubiquitin receptors are themselves ubiquitylated and that the ubiquitylation of ubiquitin receptors requires their UBDs. This is generally true for UIMs, CUE domains and GAT

domains^{15,22,26,68–71}, and has been observed for at least one NZF-motif-containing protein²⁹. It is not known whether VHS, UEV, PAZ, GLUE or UBA domains bring about the ubiquitylation of the proteins in which they are contained, although some PAZ-, UBA- and UEV-domain-containing proteins are ubiquitylated^{23,72–74}. This type of coupled ubiquitylation is probably a common, but not obligatory, feature of UBDs.

The phenomenon of coupled ubiquitin binding and ubiquitylation has been best characterized for UIM-containing proteins. UIMs generally bring about the monoubiquitylation of proteins. UIM-mediated polyubiquitylation has been found, but these experiments were carried out under conditions of ubiquitin overexpression and therefore might not reflect a physiological reaction³⁵. UIMs are necessary and sufficient for ubiquitylation because a UIM that was fused to glutathione-S-transferase or another heterologous protein resulted in the ubiquitylation of the chimeric protein⁶⁹. Ubiquitylation occurs at a site that is N-terminal to the UIM(s), not in a UIM itself^{15,35,68,69}. There is a strong link between the presence of a UBD in a protein and its ubiquitylation. The key questions now are how and why ubiquitylation is coupled to ubiquitin binding.

Mechanisms of coupled ubiquitylation. UBDs function as signals for ubiquitylation, but do not themselves contain ubiquitylation sites. It is therefore probable that UBDs promote ubiquitylation by recruiting the ubiquitylation machinery. Some ubiquitin receptors are ubiquitylated by HECT DOMAIN E3s, which form a transient thiolester bond with ubiquitin during its transfer from an E2 to a substrate (BOX 1). Because a stable interaction between UIM-containing proteins and HECT domain E3s has not been detected, Di Fiore and colleagues proposed that a UIM might bind a thiolester-linked ubiquitin that is attached to a HECT domain, rather than bind directly to the E3 (REF. 15). In this model, the E3 would then transfer the UIM-bound ubiquitin to a lysine in the UIM-containing protein. This is consistent with the structural finding that a UIM binds with its N-terminal residues close to the ubiquitin C terminus. Also in line with this model is the fact that all of the UIM mutations that are known to inhibit ubiquitylation also inhibit ubiquitin-binding. Furthermore, a mutant ubiquitin (Ile44Ala) that cannot bind UIMs cannot be conjugated to UIM-containing proteins, even though this mutant ubiquitin can undergo non-UIM-dependent conjugation³⁵. Because many E3s are themselves ubiquitylated or neddylated^{73,75,76}, another possibility is that a UIM recruits an E3 by binding to an isopeptide-linked ubiquitin or **NEDD8** on an E3 lysine. Detailed biochemical analyses of the requirements for UBD-mediated ubiquitylation are now needed to test how E3s are recruited to UBD-containing proteins.

Why are most ubiquitin receptors monoubiquitylated rather than polyubiquitylated? CUE and UBA domains partially mask Lys48 of ubiquitin, which is a primary site of chain formation (FIG. 2). Although the UIM does not sterically block Lys48 (REFS 32,43),

monoubiquitylation is still the primary type of modification that is observed for UIM-containing proteins. Perhaps UBDs that are bound to ubiquitin in *cis* generally mask a ubiquitin surface near Ile44 that is required for chain formation. Alternatively, the type of E2–E3 complex that is used in the ubiquitylation reaction might restrict the modification to monoubiquitylation, or polyubiquitin chains could be added to a ubiquitin receptor but be trimmed to monoubiquitin by a DUB³⁵. Although most UBD proteins are monoubiquitylated, specific UBDs might have the ability to promote polyubiquitylation.

Functions of coupled ubiquitylation. In most cases, the fraction of a ubiquitin receptor that is ubiquitylated in the cell is small and, as yet, few functional consequences of this modification in the cell are known. It is possible that the associated ubiquitylation is inadvertent and functionally irrelevant. However, the monoubiquitylation of the UIM-containing protein EPSIN negatively influences its *in vitro* binding to some membrane and protein partners, but not to others⁷⁷. Epsin ubiquitylation can be regulated by extracellular stimuli⁷⁷, and this ubiquitylation does not seem to function as a proteasome-degradation signal, because the protein is stable and ubiquitylated forms do not accumulate in the presence of proteasome inhibitors⁶⁹. Moreover, genetic experiments in *Drosophila melanogaster* indicate that the deubiquitylation of epsin is important for the regulation of endocytosis (REF. 78 and references therein). These observations indicate that the ubiquitylation of epsin is functionally significant. It will be important to determine whether this is the case for other ubiquitin receptors.

The ubiquitylation of ubiquitin receptors might generally have a regulatory function. Inducing an intramolecular interaction between the attached ubiquitin and the UBD could hold the UBD in an occupied position, perhaps affecting the three-dimensional conformation of the protein (FIG. 3a–c). This type of autoinhibition is reminiscent of intramolecular SH2-domain–phosphotyrosine interactions¹. Another possibility is that coupled ubiquitin binding and ubiquitylation might form a signal relay network⁷⁹. Conformational changes or the binding of a partner protein might trigger the ubiquitylation of a ubiquitin receptor, which might then lead to interactions with another downstream UBD-containing protein (FIG. 3d).

Although these are intriguing models and the functional role of ubiquitin receptor ubiquitylation needs to be defined, the necessary experiments are not trivial. It is difficult to determine whether ubiquitylation is relevant because existing mutations in UBDs abrogate both ubiquitin binding and the ubiquitylation of the ubiquitin receptor. Consequently, any phenotypic consequences of the mutation could be attributed to either function. To determine the role of UBD-dependent ubiquitylation, the ubiquitin receptor ubiquitylation site(s) must be identified, mutated to Arg, and the phenotypic consequences of this mutation determined.

HECT DOMAIN E3S (homology to E6-AP C-terminus domain E3s). These enzymes receive activated ubiquitin from an E2 and transfer it directly to a substrate protein. Unlike other E3s, these proteins have an active-site Cys residue that forms a thiolester linkage to ubiquitin during ubiquitin transfer from an E2 to a substrate.

EPSIN (Eps15-interacting protein). An essential component of the endocytic machinery that functions in cargo sorting and vesicle formation at the plasma membrane.

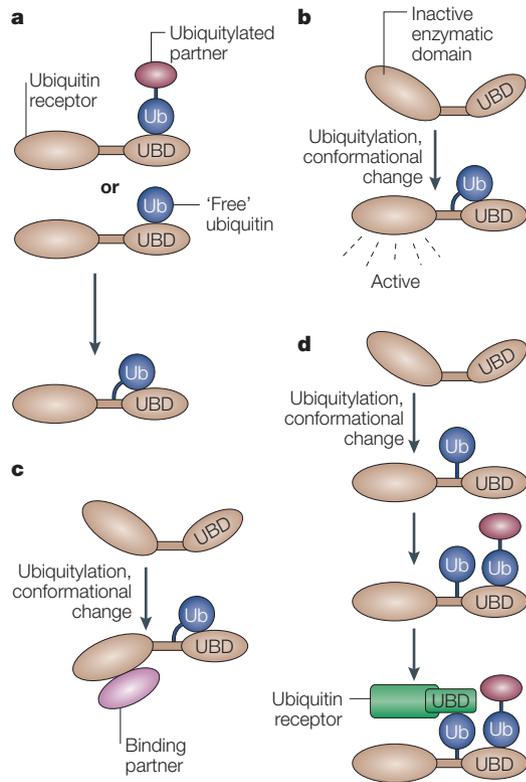


Figure 3 | Models for the function of coupled ubiquitin binding and ubiquitylation. **a** | The ubiquitylation of a ubiquitin receptor might regulate the ubiquitin-binding domain (UBD) by preventing its association with either free ubiquitin or *trans*-acting ubiquitylated partners. **b** | Alternatively, ubiquitylation might induce the recruitment of new binding partners or activate a ubiquitin receptor in other ways. The occupation of a UBD by ubiquitin that is linked in *cis* might cause a conformational change that activates an adjacent enzymatic domain. **c** | Ubiquitylation might facilitate the interaction of a protein with a binding partner by exposing previously masked binding sites. **d** | Another possibility is that monoubiquitylation might initiate a signal cascade through a ubiquitin receptor. This could occur through a conformational change that exposes a UBD such that it can recruit another ubiquitylated protein or through the subsequent binding of a second ubiquitin receptor. Several variations on these themes are possible. The actual function of coupled ubiquitin binding and ubiquitylation needs to be defined and might differ from protein to protein. Ub, ubiquitin.

UBL-UBA PROTEINS
A family of proteins that carry an N-terminal ubiquitin-like (Ubl) domain and one or two ubiquitin-associated (UBA) domains. Some members are involved in the proteasome-mediated degradation of ubiquitylated proteins.

SORTING SIGNAL
This is usually a linear amino acid sequence or a post-translational modification that specifies the cellular location or destination of an integral membrane protein.

SECRETORY PATHWAY
The path (which is typically the endoplasmic reticulum (ER) to the Golgi to the lysosome or plasma membrane) that is followed by proteins that enter the ER during translation. This pathway transports proteins to their ultimate cellular or extracellular locations.

Physiological roles of ubiquitin-binding domains
UBDs are found in proteins that function in a vast range of cellular events. The various different UBDs and the numerous contexts in which they are found are probably necessary to achieve the many different outcomes that can take place in response to ubiquitylation. In the case of phosphorylation, the effects of adding a simple phosphate group to a protein can be numerous and profound; ubiquitylation offers even more possibilities. Although our understanding of how UBDs mediate biological responses is in its infancy, three of the best-understood examples so far are discussed below.

UBDs in ubiquitylation and deubiquitylation machinery.
UBDs are found in several components of the ubiquitylation machinery and in many deubiquitylating enzymes (see SMART in the Online links box). For at least one E2, a molecular structure and mutagenesis studies support a role for a UBD in polyubiquitin-chain formation. Specifically, a structural model of the Mms2-ubiquitin-conjugating enzyme-13 (Ubc13) E2 complex indicates that a UEV domain might function as a substrate-binding domain in the formation of Lys63-linked chains⁶¹, chains which have non-proteolytic functions in kinase activation and DNA repair. The Mms2 UEV domain binds to ubiquitin non-covalently and orients the ubiquitin molecule such that Lys63 is placed near the Ubc13 active site, where it can form a bond with the C terminus of a Ubc13-linked activated ubiquitin. In this position, Lys48 of the Mms2-bound ubiquitin is masked, which is consistent with the specific linking to Lys63 that is carried out by this complex^{61,62}. So, in this case, the ubiquitin-binding UEV domain in Mms2 functions as an orientation-specific ubiquitin-binding module that determines the chain-forming specificity of an E2.

Although many deubiquitylation enzymes have one or more UBDs, the roles of these domains in deubiquitylation reactions have not been defined. As for E2s, the DUB UBDs might be involved in substrate recognition or they might function as regulators of enzyme activity.

Ubiquitin receptors in proteasomal degradation. Polyubiquitylated proteins are targeted to the proteasome by ubiquitin-binding proteins (for a review, see REF. 40). However, contrary to the long-held assumption that there would be a single proteasomal receptor for all polyubiquitylated proteins, increasing evidence indicates that there are several receptors that each link a subset of proteins to the proteasome^{80,81}. Some of these receptors are stable subunits of the proteasome, whereas others associate with the proteasome transiently. Stably associated subunits that bind to polyubiquitin chains include the UIM-containing protein S5a/Rpn10 and an ATPase that lacks a defined UBD^{82,83}. UBL-UBA PROTEINS that are transiently associated with the proteasome are examples of shuttling receptors. The Ubl domains in these proteins bind to the proteasome, which brings the polyubiquitylated proteins that are bound to the UBA domains to their site of destruction⁸⁴. The UBA domains in these ubiquitin receptors have a relatively high affinity for Lys48-linked polyubiquitin chains ($K_d = 0.03-9 \mu\text{M}$; see TABLE 1) and are substrate specific⁸¹, so the Ubl-UBA proteins might recognize binding determinants in both the conjugated protein and ubiquitin. There might also be other types of ubiquitin-binding shuttling factor that have distinct substrate specificities and different target destinations.

Ubiquitin receptors in the endocytic pathway. Mono-ubiquitin functions as a cargo SORTING SIGNAL at several locations in the SECRETORY PATHWAY and endocytic pathway. Furthermore, a number of proteins that

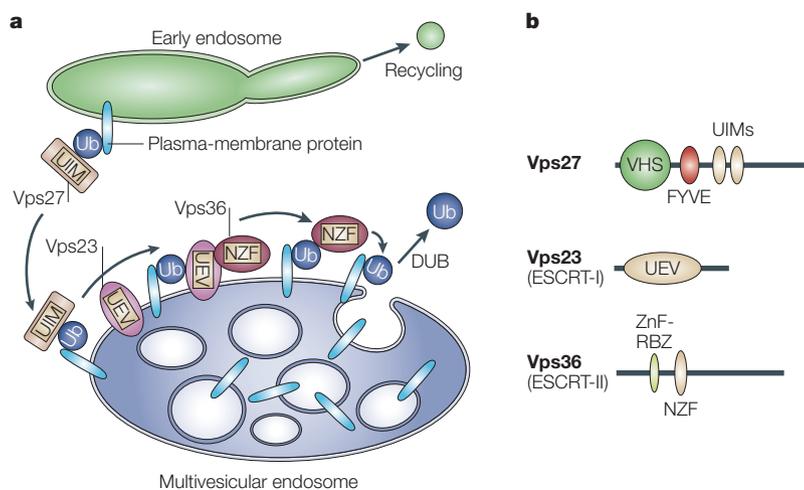


Figure 4 | The proposed role of ubiquitin receptors in the late endosomal pathway. Depicted is the probable role of ubiquitin-binding domains in cargo sorting into the luminal vesicles of yeast multivesicular endosomes/bodies (MVEs/MVBs). A similar pathway that uses homologous proteins functions in mammalian cells. **a** | Using its ubiquitin-interacting motif (UIM), vacuolar protein sorting-27 (Vps27) recognizes ubiquitylated plasma-membrane proteins that have been internalized into an early endosomal compartment. This recognition prevents cargo from entering a recycling pathway and instead sorts cargo to an MVE. Here, three protein complexes, which are known as endosomal sorting complex required for transport-I (ESCRT-I), -II and -III, function sequentially to move ubiquitylated cargo into vesicles that bud into the endosomal lumen (for clarity, only the ubiquitin-binding subunits of the ESCRTs are shown). In the present model, Vps27 transfers ubiquitylated cargo to the UEV (ubiquitin-conjugating enzyme variant) domain of Vps23, a component of ESCRT-I. Vps23 then passes the cargo to the NZF (Npl4 zinc finger) domain of Vps36, which is a subunit of ESCRT-II. Ubiquitin-binding protein components have not yet been identified in the ESCRT-III complex. The cargo is deubiquitylated by the action of a deubiquitylating enzyme (DUB), before being sequestered in an MVE vesicle. **b** | The domain structures of the ubiquitin receptors that are required for cargo sorting into MVE vesicles. Vps36 carries two related ZnF-RBZ sequences (zinc finger domain in Ran-binding and other proteins), one of which is an NZF motif, which carries the crucial Thr-Phe motif that is required for ubiquitin binding. FYVE 'Fab1, YOTB, Vac1, EEA1'; Ub, Ubiquitin; VHS 'Vps27, HRS, STAM'.

are required in the endocytic pathway are mono-ubiquitylated, and several observations indicate that ubiquitylation regulates the activity of the endocytic machinery (for a review, see REF. 6). Consistent with the multiple roles of ubiquitin in endocytosis, numerous UBDs are also required in this process^{16,17,25,57,65,85}. Because both the cargo and the transport machinery are ubiquitylated, it has been difficult to delineate the specific functions of the UBDs of endocytic proteins. However, at one step in the pathway there is a straightforward, although as-yet-unproven, model for the role of UBDs as receptors for ubiquitylated cargo (FIG. 4a).

Transmembrane proteins from both the secretory and endocytic pathways are targeted to the interior of the LYSOSOME by a sorting step that occurs at a late endosomal compartment known as the MULTIVESICULAR ENDOSOME/BODY (MVE/MVB)^{86,87}. Here, transmembrane proteins are sorted into vesicles that bud into the lumen of the MVE. After the fusion of the MVE with a lysosome, the vesicles and associated proteins are delivered to the lysosome lumen and degraded. Transmembrane proteins can be sorted into MVE vesicles by a monoubiquitin signal

that is attached to their cytoplasmic domain^{48,88}. This ubiquitin signal might be recognized by three proteins that are proposed to function sequentially in the sorting and vesicle-budding pathway^{14,16,17,33}. Each of the three proteins has a different UBD that is important for function (FIG. 4b), which indicates that ubiquitylated cargo might be passed from one ubiquitin receptor to another along the pathway⁶⁵. To prove this model, the direct binding of ubiquitylated cargo to the ubiquitin receptors and the transfer of ubiquitylated cargo from protein to protein needs to be shown. Another possible function for UBDs in MVE sorting is in promoting protein-protein interactions among the sorting machinery itself.

The three mechanisms described above are the best examples we have for how UBDs might function in various cellular events. UBDs are required in many other processes, including for the activation of kinases in the nuclear factor- κ B signalling pathway²⁹ and for membrane fusion during organelle reassembly after mitosis²⁸. We look forward to learning more about how ubiquitin-UBD interactions regulate different cellular phenomena.

What next?

There are nine characterized UBDs so far (TABLE 1), and there are certainly more to come. This prediction is supported by the high degree of amino-acid sequence conservation on all of the surfaces of ubiquitin and by reports of unidentified ubiquitin-binding sites in several proteins that are associated with ubiquitin-dependent processes (for example, see REFS 27,89–91). Distinct binding motifs for the Ubl SUMO were recently described^{92–94}, and at least one SUMO-binding sequence is required for the sumoylation of the protein in which it is contained⁹⁴. These observations indicate that there are domains that are analogous to UBDs for other members of the Ubl family.

Although we have molecular models of how ubiquitin binds to many of the characterized domains, we do not generally understand why some UBDs do not bind ubiquitin or why some show a preference for a particular ubiquitin modification. In addition, we need to further understand the regulation of ubiquitin binding by UBDs in the context of full-length proteins, as well as the relationship between UBDs and coupled ubiquitylation. Finally, we understand little about the functional aspects of UBDs in the context of full-length proteins. Similar to phosphorylation, ubiquitylation functions in numerous pathways and can elicit various biochemical responses. We need to know how UBDs regulate protein location and activity if we are to fully understand the various ways in which ubiquitin signals control biological processes.

Note added in proof

Cadwell and Coscoy have shown that the ubiquitylation of substrate proteins in cells can occur on cysteine thiol groups¹⁰⁴.

LYSOSOME

An acidic organelle that contains digestive enzymes. In this organelle, proteins and other cellular molecules are degraded into their building-block subunits (for example, proteins into amino acids).

MULTIVESICULAR ENDOSOME/BODY

An organelle in the late endocytic pathway that is filled with vesicles that have budded from the limiting membrane into the lumen. Fusion of a multivesicular endosome with a lysosome delivers the vesicles into the lysosome lumen, where they are degraded.

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