Ubiquitination

The central role of the Ubiquitin Proteasome Pathway (UPP) in biology has been recognized with the Nobel Prize for Chemistry which was awarded to Avram Hershko, Aaron Ciechanover and Irwin Rose in 2004.

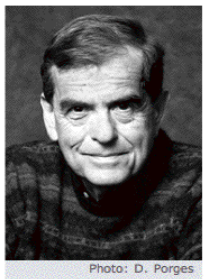


Photo: D. Porges

Aaron Ciechanover

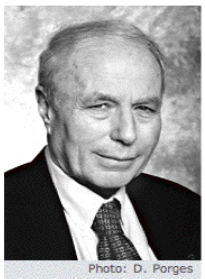
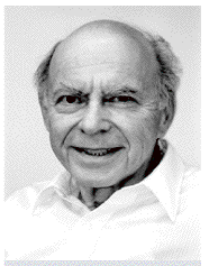


Photo: D. Porges

Avram Hershko



Irwin Rose

1

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 an intracellular signal that is inducibly and reversibly attached to a range of proteins and, as such, regulates a multitude of cellular functions.

Ubiquitin has a diverse surface architecture and forms differently coupled chains, thus expanding its capacity to act as a versatile signalling messenger.

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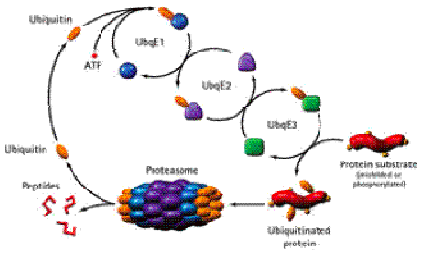
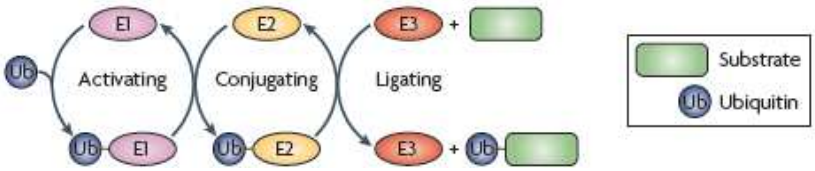
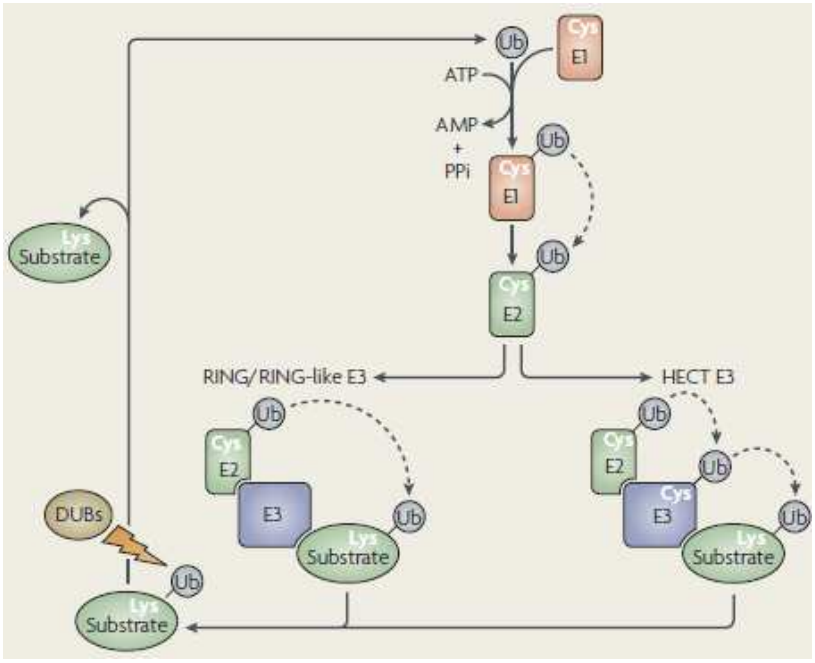
Historically, the first role of Ubiquitin is in UPP

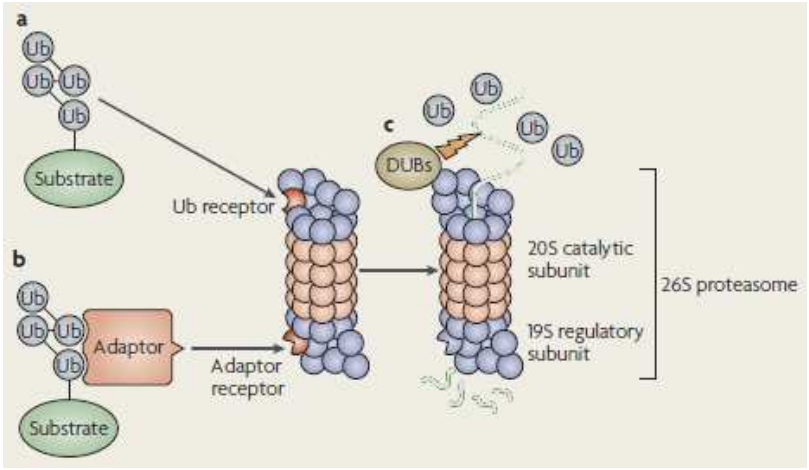
Ubiqutin-Proteasome Pathway

Degradation of a protein via the UPP involves two discrete and successive steps: tagging of the substrate protein by the covalent attachment of multiple ubiquitin molecules (conjugation), and the subsequent degradation of the tagged protein by the 26S proteasome (composed of the catalytic 20S core and the 19S regulator). This classical function of ubiquitin is associated with housekeeping functions, regulation of protein turnover and antigenic-peptide generation.

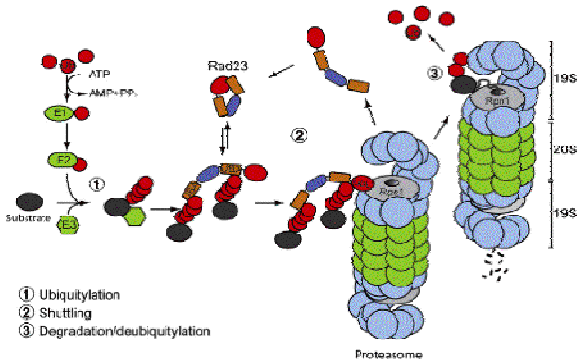








Once modified by a polyubiquitin chain of at least four ubiquitins (Ub), the substrate protein can bind either directly to intrinsic Ub receptors in the 19S regulatory complex of the 26S proteasome (see figure panel **a) or to adaptor proteins that contain both** poly ubiquitin-binding and proteasome-binding domains (see figure panel **b). It is not** fully understood why certain polyubiquitin-modified substrates must be shuttled to the proteasome by adaptor proteins and others can associate directly with polyubiquitinbinding subunits in the proteasome regulatory complex. Binding of the substrate protein to the proteasome is followed by protein unfolding by the half-dozen ATPases (not shown) that encircle the pore of the proteasome catalytic core, removal of the polyubiquitin chain by proteasome-associated deubiquit-ylating enzymes (DUBs), and translocation of the unfolded protein into the central proteolytic chamber, where it is cleaved into short peptides (see figure panel **c).**



The role of Rad23 in ubiquitin-dependent proteasomal degradation. The UPS can be divided in ubiquitylation (left panel) and proteasomal degradation (right panel). Proteins harboring a degradation signal are ubiquitylated by an enzymatic cascade consisting of a ubiquitin activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), a ubiquitin ligase (E3) and, optionally, a ubiquitin chain elongation factor (E4). The polyubiquitylated protein binds directly to the proteasome or, alternatively, binds to the UBA domains of Rad23 or other ubiquitin receptors (Dsk2, Ddi1). The UbL domain of Rad23 binds to the Rpn1 subunit in the 19S regulator of the proteasome. Rad23 resists proteasomal degradation and is released from the proteasome. The polyubiquitylated substrate is deubiquitylated, unfolded and degraded in the 20S core particle of the proteasome. 4

UV excision repair protein Rad23

The UPP is central to the regulation of almost all cellular processes including:

- Antigen processing
- Apoptosis
- Biogenesis of organelles
- Cell cycle and division
- DNA transcription and repair
- Differentiation and development
- Immune response and inflammation
- Neural and muscular degeneration
- Morphogenesis of neural networks
- Modulation of cell surface receptors, ion channels and the secretory pathway
- Response to stress and extracellular modulators
- Ribosome biogenesis
- Viral infection

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Ubiquitin chains — diverse cellular signals.

monoubiquitylation	Alters protein activity and localization (by regulating endocytosis, lysosomal targeting, meiosis and chromatin remodelling).
polyubiquitylation	The formation of a diverse array of ubiquitin chains is implicated in events such as targeting to the 26S proteasome, immune signalling and DNA repair. The linear ubiquitin chain assembly complex (LUBAC) and are crucial for nuclear factor-B (NF-B) signalling

2002 Review

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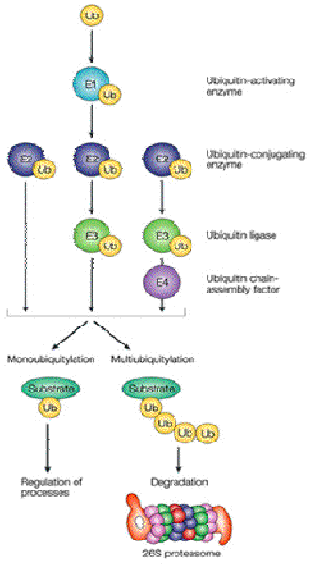
The ubiquitin pathway.

Free ubiquitin (Ub) is activated in an ATP-dependent manner by the activity of a ubiquitin-activating enzyme (E1), which hydrolyses ATP and forms a complex with ubiquitin. Subsequently, ubiquitin is transferred to one of many distinct ubiquitin-conjugating enzymes (E2s). In some reactions, E2s can directly ubiquitylate substrates, whereas others require the help of ubiquitin ligases (E3s). Some E3s function catalytically (homologous to E6AP carboxyl-terminus (HECT)-type E3s; as shown), whereas other E3s, including RING-finger proteins and SCF and SCF-like complexes, support ubiquitylation by recruiting substrates to the ubiquitylating enzymes. Usually, several ubiquitin molecules, in the form of a multiubiquitin chain, are conjugated to a substrate. This reaction sometimes requires a specific multiubiquitin chain assembly factor (E4). Multiubiquitylation serves mainly, but not exclusively, to label the substrate for degradation, whereas monoubiquitylation regulates several processes, such as endocytosis, DNA repair and transcriptional regulation.

Deadly encounter: ubiquitin meets apoptosis
Vercelia Jesenberger and Stefan Jentsch
Nature Reviews Molecular Cell Biology **3**, 112-121 (February 2002)



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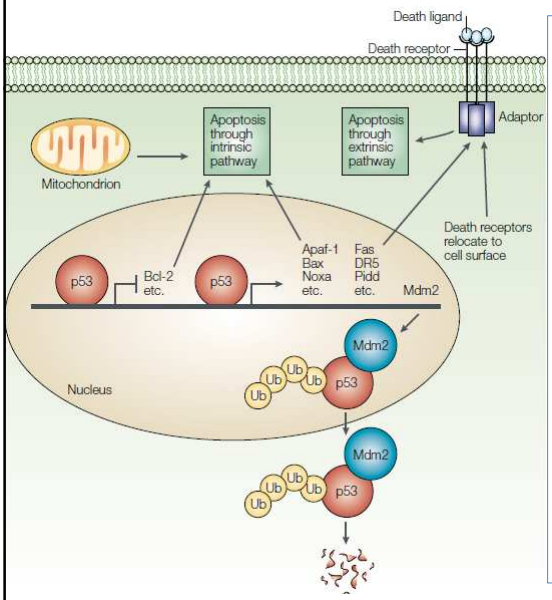
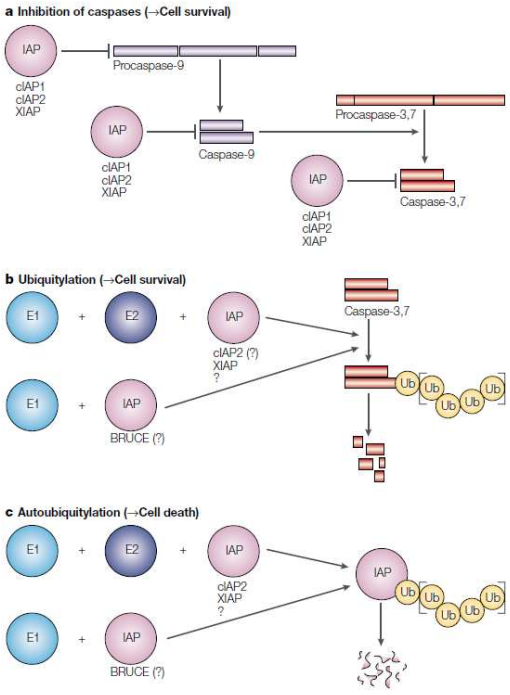
apoptosis



p53 and apoptosis. p53 induces the expression of proteins that target both the mitochondrial- and the death-receptor-induced apoptotic pathways, and specifically represses transcription from several death-inhibiting genes. Further activities of p53 that are entirely independent of transcriptional regulation have been proposed. They include the ability of p53 to drive relocalization of death receptors such as Fas/CD95 from the Golgi to the cell surface and to directly associate with mitochondria. Central to the regulation of p53 is Murine double minute 2 (Mdm2), which itself is a transcriptional target of p53. Mdm2 binds to p53 and targets p53 for ubiquitin/proteasome-dependent degradation. Ubiquitylation (Ub) of p53 by Mdm2 probably also enhances the export of p53 from the nucleus to the cytoplasm, where degradation takes place. Bcl-2, B-cell lymphoma 2; Apaf, Apoptotic protease-activating-factor; Bax, Bcl-2 associated X protein; DR5, death receptor 5; Pidd, p53 protein induced, with death domain.

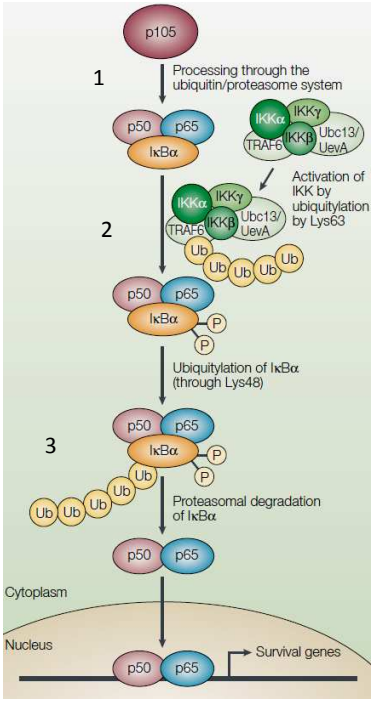


The multiple roles of IAPs. X-linked inhibitor of apoptosis (XIAP), cIAP1, and cIAP2 can directly bind to activated caspases and inhibit their activities. In addition, they interact with procaspase-9 and prevent its activation by apoptotic stimuli. The RING-finger proteins XIAP and cIAP2 have been shown to promote the ubiquitylation of activated effector caspases. The E3 activity of XIAP targets caspase-3 for degradation, and thereby enhances the inhibitory effect of XIAP on apoptosis. It is tempting to speculate that, analagous to the ubiquitin ligase XIAP, the BIR-repeat-containing ubiquitin-conjugating enzyme (BRUCE) can also transfer ubiquitin (Ub) to caspases. The E3 ubiquitin ligases XIAP and cIAP1 are ubiquitylated and degraded by proteasomes in response to apoptotic stimuli in T cells, and their degradation seems to be important for T cells to commit to death. IAP, inhibitor of apoptosis protein.

The NF-κB pathway. Nuclear factor κB (NF-κB) is a collective name for inducible dimeric transcription factors that are composed of members of the Rel family of DNA-binding proteins. Five mammalian Rel proteins have been identified: NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), c-Rel, RelA (p65) and RelB (REF. 65).

1° step

Strikingly, the ubiquitin/proteasome system is involved in at least three steps that are essential for the activation of NF-κB. First, the NF-κB1 and NF-κB2 genes encode precursor proteins that are much larger — 105 kDa and 100 kDa — than the mature functional proteins, p50 and p52, respectively. The precursors must be processed to generate the mature forms. Although

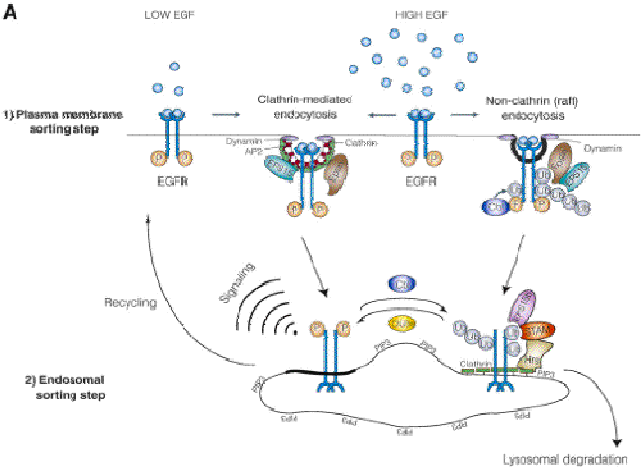


Control of NF-κB activity by the ubiquitin/proteasome system. The most classical form of nuclear factor-κB (NF-κB) is a heterodimer of p50 and p65. The precursor form of p50, p105, is processed in a ubiquitin/proteasome-dependent manner to its mature form. p50 is present in the cytoplasm as a dimer with p65, and associated with an inhibitor of NF-κB such as IκBα. By binding to NF-κB, IκBα masks its nuclear localization signal, thereby preventing nuclear uptake. Following stimulation of cells by various agonists, IκBα is rapidly phosphorylated by the IκB kinase (IKK) complex. IKK itself is activated by ubiquitylation (Ub) (not linked to proteolysis) which involves tumour-necrosis factor (TNF)-receptor associated factor 6 (TRAF6), a RING-finger protein that collaborates with the heterodimeric Ubc13/Uev1A ubiquitin conjugating enzyme complex (also known as TRAF6-regulated IKK activator 1 (TRIKA-1)) in the synthesis of Lys63-linked multiubiquitin chains. The target of this unusual modification seems to be TRAF6 itself. After phosphorylation by activated IKK, the phosphoacceptor sites on IκBα serve as an essential part of a specific recognition site for the ubiquitin ligase RSIκB/β-TrCP, and IκBα is rapidly ubiquitylated and degraded by the proteasome. Following IκBα degradation, NF-κB translocates to the nucleus where it regulates the expression of a wide spectrum of genes that are involved in immunity, inflammation, apoptosis and other cellular processes.

2° pathway: sorting to the lysosomal comparment

Multiple sorting steps control EGFR and TGFβR trafficking and signaling.

A



(A) The first sorting step is at the plasma membrane, where EGFR can be internalized through different endocytic pathways as a function of EGF dose. In the clathrin route, receptors are mostly directed to recycling and signaling, while in the non-clathrin route they are preferentially targeted for lysosomal degradation. A second sorting step is present at the level of the endosomes, where the two internalization pathways seem to converge.

A flat clathrin lattice on the endosomal membrane stabilizes the interaction between the ESCRT-0 complex (Hrs, STAM and Eps15b) and ubiquitinated EGFR, which is then targeted for degradation. Counteraction between DUBs (possibly AMSH) and Cbl is shown.

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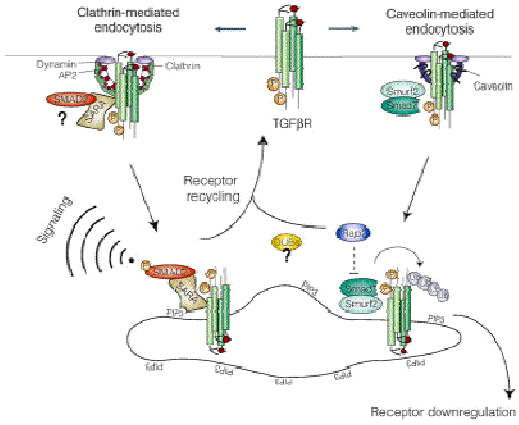
Multiple sorting steps control TGFβR trafficking and signaling.

B



(B) TGFβRs internalized through clathrin-mediated endocytosis are directed towards the early endosomes (enriched in PIP3, phosphatidylinositol-3-phosphate). Here, interaction with the SARA/Smad2 complex allows signaling and recycling.

In the caveolar pathway, TGFβRs associate with the SMAD7–SMURF2 complex, which targets receptors for ubiquitin-dependent degradation. In this route, TGFβRs reach a yet undefined compartment, which is in dynamic communication with the "SARA signaling endosome". A second sorting step at this level is exerted by the small-GTPase Rap2, which counteracts the action of Smad7, leading to delayed receptor degradation and increased signaling/recycling.

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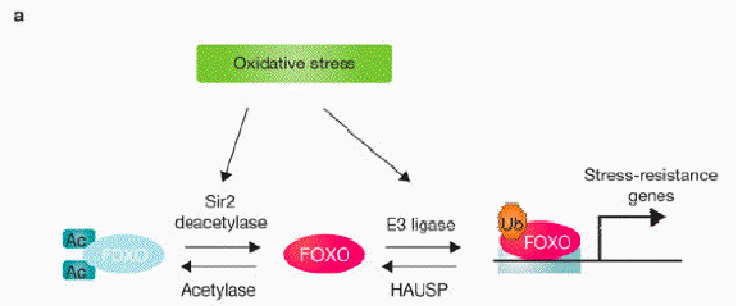
Mono-ubiquitination is often a localization signal

Regulation of FOXO by monoubiquitination and deubiquitination.



(a) Under oxidative conditions, FOXO transcription factor is deacetylated to promote its monoubiquitination by an unknown ubiquitin E3 ligase. **Monoubiquitinated FOXO accumulates in the nucleus to activate its target gene transcription.** Oxidative stress also, either directly or indirectly, enhances the binding of the DUB-HAUSP to FOXO. This, in turn, allows sequential deubiquitination of FOXO to curtail its transcriptional response towards oxidative stress.

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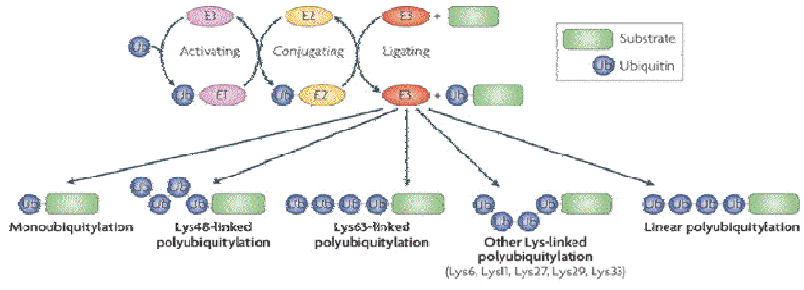
1) quali differenti modificazioni esistono?

2) quali proteine riconoscono queste modificazioni?

3) come vengono riconosciute le proteine alle quali apportare determinate modificazioni?

Una review recente indica una situazione complicata per l'Ubiquitinazione

Enzymatic cascade that leads to substrate ubiquitylation.



The activity of three enzymes is required for ubiquitylation: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-ligating enzyme (E3), which recognizes the substrate. The completion of one ubiquitylation cycle results in a monoubiquitylated substrate. However, the cycle can be repeated to form polyubiquitylated substrates.
Ubiquitin can be covalently attached to target proteins as a single moiety (monoubiquitin), as multiple single moieties (multiple monoubiquitin), as chains coupled through the same Lys residue in ubiquitin (homotypic polyubiquitin), as mixed chains linked through different Lys residues in ubiquitin (branched polyubiquitin) or as head-to-tail bound ubiquitin moieties (linear polyubiquitin).

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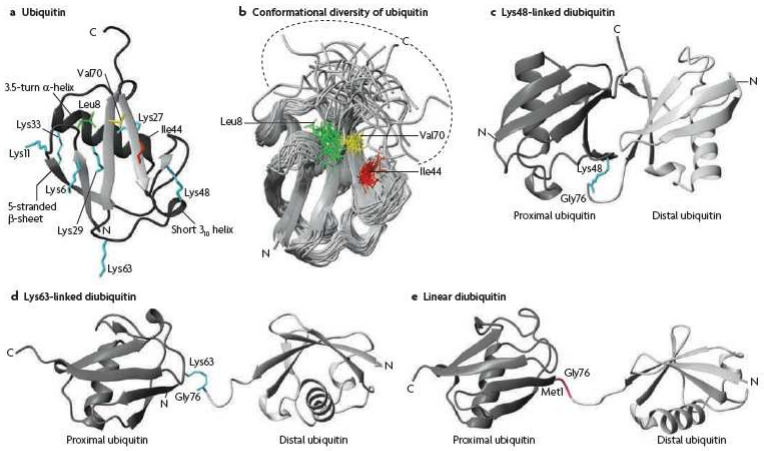

Figure 3 | **Structural diversity contributes to the multiplicity of ubiquitin signalling. a** | A ribbon representation of monoubiquitin (Protein Data Bank (PDB) identifier 1D3Z)[107]. Ubiquitin contains a 5-stranded β-sheet, a 3.5-turn α-helix and a short 3_{10} helix. Seven solvent-exposed Lys residues (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. **b** | Free ubiquitin in solution is a dynamic molecule with conformational diversity. Distinct conformations are selected by individual UBDs and several are shown here to highlight the dynamic range of motions that ubiquitin displays in solution[9]. **c** | Ribbon representation of Lys48-linked diubiquitin (PDB identifier 1AAR)[12]. Lys48-linked chains form compact structures as a result of inter-moiety interactions. The isopeptide bond linkage is shown in cyan. **d** | Ribbon representation of Lys63-linked diubiquitin (PDB identifier 2JF5). The isopeptide bond linkage is shown in cyan. **e** | Ribbon representation of linear diubiquitin, forming a peptide bond (magenta) between Met1 and Gly76 (PDB identifier 2W9N)[11]. Lys63-linked and linear ubiquitin chains have more extended conformations than Lys48-linked chains. Linkage of ubiquitin molecules into a polymer enhances the structural diversity for robust signalling. In each case, the linker and its neighbouring region are chemically diverse.

Although ubiquitin is the most well understood post-translation modifier, there is a growing family of ubiquitin-like proteins (UBLs) that modify cellular targets in a pathway that is parallel to but distinct from that of ubiquitin. These alternative modifiers include: SUMO (Sentrin, Smt3 in yeast), NEDD8 (Rub1 in yeast), ISG15 (UCRP), APG8, APG12, FAT10, Ufm1 URM1 & Hub1.

These related molecules have novel functions and influence diverse biological processes. There is also cross-regulation between the various conjugation pathways since some proteins can become modified by more than one UBL, and sometimes even at the same lysine residue. For instance, SUMO modification often acts antagonistically to that of ubiquitination and serves to stabilize protein substrates. Proteins conjugated to UBLs are typically not targeted for degradation by the proteasome, but rather function in diverse regulatory activities. Attachment of UBLs might alter substrate conformation, affect the affinity for ligands or other interacting molecules, alter substrate localization and influence protein stability.

Ubiquitin-like molecules

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More recently, it has become evident that protein modification by ubiquitin also has unconventional (non-degradative) functions such as the regulation of DNA repair and endocytosis. These non-traditional functions are dictated by the number of ubiquitin units attached to proteins (mono- versus poly-ubiquitination) and also by the type of ubiquitin chain linkage that is present.

Ubiquitin becomes covalently linked to itself and/or other proteins either as a single molecule or as poly-ubiquitin chains. The attachment of ubiquitin to the ε-amine of lysine residues of target proteins requires a series of ATP-dependent enzymatic steps by E1 (ubiquitin activating), E2 (ubiquitin conjugating) and E3 (ubiquitin ligating) enzymes. The C-terminal Gly75-Gly76 residues of ubiquitin are the key residues that function in the diverse chemistry of ubiquitin reactions. Ubiquitin can be conjugated to itself via specific lysine (K6, K11, K27, K29, K33, K48 or K63) residues which results in diverse types of chain linkages. These covalent ubiquitin bonds (isopeptide linkages) can be reversed by specific deubiquitinating enzymes which remove ubiquitin conjugates from proteins and disassemble ubiquitin chains.biquitin chains.

9

Ubiquitin acts as a signalling component that can trigger molecular events in cells. It does this by operating as a reversible and highly versatile regulatory signal for **ubiquitin-binding domains (UBDs)** in cellular proteins, new varieties of which are still being discovered. Many molecular details of signal transmission from ubiquitylated proteins (substrates that are modified following various cellular stimuli) to effector proteins (ubiquitin receptors containing one or more UBDs) have been elucidated in the past decade

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 to these functions.

These preferences originate from multimeric interactions, whereby UBDs synergistically bind multiple ubiquitin molecules, 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. These new structure-based insights provide strategies for controlling cellular processes by targeting ubiquitin–UBD interfaces.

10

Ubiquitin binding domains

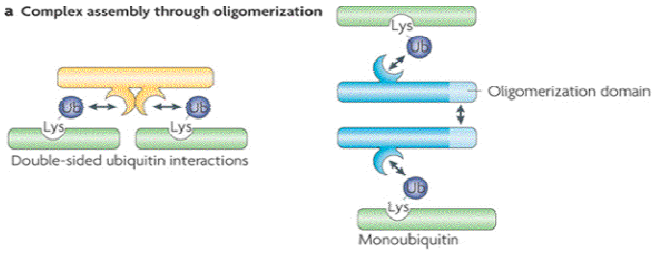
Ubiquitin-binding domains — from structures to functions

Ivan Dikic[*‡§], *Soichi Wakatsuki*[‖] *and Kylie J. Walters*[¶]

Abstract | 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 to these functions. These preferences originate from multimeric interactions, whereby UBDs synergistically bind multiple ubiquitin molecules, 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. These new structure-based insights provide strategies for controlling cellular processes by targeting ubiquitin–UBD interfaces.

NRMCB 2009

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 substrates. Alternatively, two proteins carrying oligomerization domains and UBDs can indirectly bridge the same ubiquitylated substrate. In both cases this results in the formation of protein complexes, which might help to amplify a signal or activate a downstream process.

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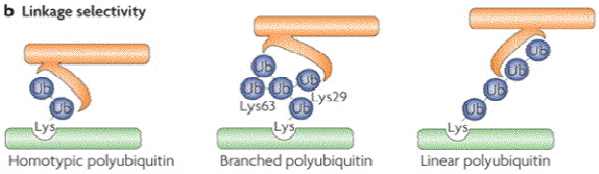
b | Specialized UBDs have also been discovered that can selectively discriminate between different types of ubiquitin chains.



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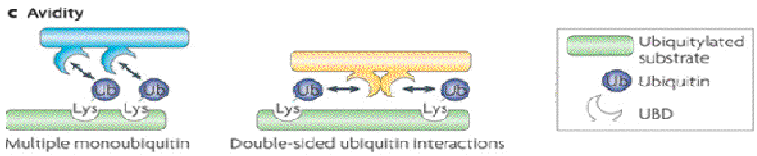

c | The presence of two or more UBDs in a protein or the attachment of multiple ubiquitin moieties on the same substrate can increase the avidity of and promote ubiquitin–UBD interactions, despite the low affinities of the individual interactions. This phenomenon might be important to filter noise coming from nonspecific transient ubiquitin–UBD interactions and to amplify only the output of proper ubiquitin–UBD pairs. UBDs that bind to one ubiquitin moiety are shown in blue, those that interact specifically with the regions linking ubiquitin moieties are in orange, and double-sided UBDs are in yellow. Arrows indicate protein–protein interactions.

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Table 1 | **The functional and structural diversity of ubiquitin-binding domains**

Ubiquitin-binding domain	Representative protein*	Function	References
α-Helix			
UIM	S5a (human) and Rpn10 (yeast), Vps27, STAM, epsins and RAP80 (UIMC1)	Proteasome degradation, endocytosis, MVB biogenesis and DNA repair	26,27,77,110
IUIM (also known as MIU)	RABEX5	Endocytosis	24,25
DUIM	HRS	MVB biogenesis	23
UBM	Polymerase iota and reversionless 1	DNA damage tolerance	38
UBAN	NEMO, ABIN1–ABIN3 and optineurin	Nuclear factor-κB signalling	8,11,50,51
UBA	Rad23 (yeast) and R23A (human), Dsk2 and NBR1	Proteasome targeting, kinase regulation and autophagy	30,42,111, 112
GAT	GGA3 and TOM1	MVB biogenesis	58,60
CUE	Vps9, TAB2 and TAB3	Endocytosis and kinase regulation	29,113
VHS	STAM and GGA3	MVB biogenesis	114
Zinc finger (ZnF)			
UBZ	Polymerase-h; polymerase-k and Tax1BP1	DNA damage tolerance and nuclear factor-κB signalling	38,115
NZF	NPL4, Vps36, TAB2 (MAP3K7IP2) and TAB3 (MAP3K7IP3)	ERAD, MVB biogenesis and kinase regulation	37,116,117
ZnF A20	RABEX5 (RABGEF1) and A20 (TNFAIP3)	Endocytosis and kinase regulation	24,25
Znf UBP (also known as PAZ)	Isopeptidase T (USP5) and HDAC6	Proteasome function, aggresome function and autophagy	39,118
Plekstrin homology (PH) domain			
PRU	RPN13	Proteasome function	35,36
GLUE	EAP45 (VPS36)	MVB biogenesis	35,36
Ubiquitin-conjugating (Ubc)-like domain			
UEV	UEV1 (UBE2V1) and MMS2	DNA repair, MVB biogenesis and kinase regulation	119,120
UBC	UBCH5C (UBE2D3)	Ubiquitin transfer	32
Others			
SH3	Sla1 and CIN85 (SH3KBP1)	Endocytosis	121
PFU	Ufd3 (Doa1)	ERAD	122
Jab1/MPN	Prp8	RNA splicing	123

1. UBDs that bind monoubiquitin (UIM, IUIM, MIU, UBZ)
2. Lysine-linkage-specific UBDs (Rad23-K48; UBA-domain: K63; Tab2-NZF: KK63)
3. UBDs specific for linear ubiquitin chains (Met-Gly)
4. Multivalent ubiquitin-UBD interactions

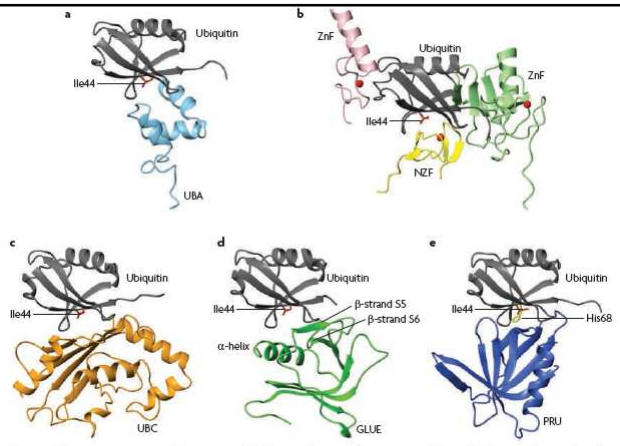

Figure 4 | **Ubiquitin is recognized by structurally diverse domains.** The structures of several ubiquitin–ubiquitin-binding domain (UBD) complexes. Ubiquitin (grey) is shown in the same orientation to highlight the common use of its β-strand surface by diverse UBDs. **a** | Ribbon representation of ubiquitin in complex with the ubiquitin-associated (UBA) domain of protein linking IAP with cytoskeleton 1 (PLIC1; light blue) (Protein Data Bank (PDB) indentifier 2JY6)[108]. **b** | Ribbon representation of ubiquitin in complex with the A20-type zinc finger (ZnF) domain (yellow) of RAB5 guanine nucleotide exchange factor (RABEX5; also known as RABGEF1; light green) (PDB indentifier 2FIF)[34], the nuclear protein localization 4 (NPL4) ZnF (NZF) domain (PDB indentifier 1Q5W)[37] and the ZnF domain of the deubiquitinase (DUB; also known as deubiquitylating or deubiquitinating enzyme) isopeptidase T ubiquitin-binding protein (UBP; pink) (PDB indentifier 2G45)[38]. The NZF domain binds to the Ile44-centred surface, the A20 ZnF domain of RABEX5 binds to an Asp58-centred surface and the ZnF domain of isopeptidase T UBP binds to the carboxyl terminus. Zinc atoms are shown as red spheres. **c** | Ribbon representation of ubiquitin in complex with the E2 ubiquitin-conjugating enzyme UBCH5C (also known as UBE2D3; orange) (PDB indentifier 2FUH)[32]. **d** | Ribbon representation of ubiquitin in complex with the GRAM-like ubiquitin-binding in EAP45 (GLUE) domain of ELL-associated protein of 45 kDa (EAP45; also known as VPS36; dark green) (PDB identifier 2DX5)[33]. **e** | Ribbon representation of ubiquitin in complex with the plekstrin homology for ubiquitin (PRU) domain of regulatory particle, non-ATPase-like 13 (RPN13; dark blue) (residues 1–150; PDB identifier 2Z59)[35]. The side chain atoms of His68 are shown in yellow. The three-helix bundle structure of the UBA, E2 ubiquitin-conjugating and plekstrin homology domains binds to the Ile44-centred hydrophobic patch in ubiquitin, but does so in diverse manners and cannot simultaneously act on a common monoubiquitin or ubiquitin moiety within a chain. By contrast, ZnF domains

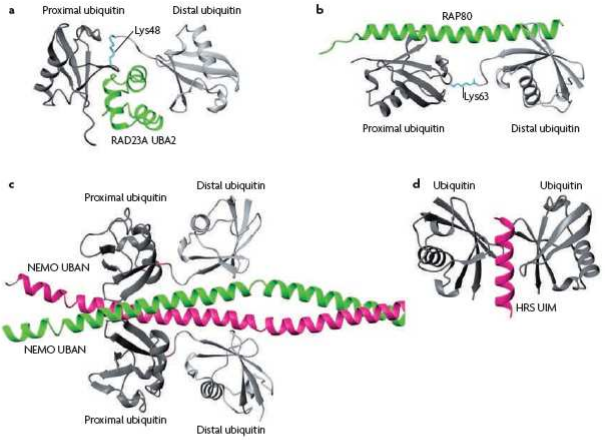

Figure 5 | **Multivalent interactions between ubiquitin and UBDs define chain specificity and increase affinity.** **a** | The ubiquitin-associated 2 (UBA2) domain (green) of a radiation sensitivity abnormal 23 human homologue, RAD23A, is sandwiched between the two ubiquitin moieties of Lys48-linked diubiquitin (grey) (Protein Data Bank (PDB) indentifier 1ZO6)[43]. This structure provides an explanation for the preference of this domain for Lys48-linked chains: it contacts the ubiquitin linker region to expand its binding surface beyond that possible for monoubiquitin. **b** | Structure of Lys63-linked diubiquitin bound to receptor associated protein 80 (RAP80), illustrating why RAP80 binds to Lys63-linked, but not Lys48-linked, chains (coordinates provided by S. Fukai, University of Tokyo, Japan)[109]. Its contiguous helix binds simultaneously to two ubiquitin moieties, thereby increasing its affinity beyond that possible with monoubiquitin and making the spacing between the two ubiquitin moieties greater than would be achievable with the Lys48 linkage. **c** | The UBAN (ubiquitin binding in ABINs (A20-binding inhibitor of nuclear factor-κB proteins) and NEMO (nuclear factor-κB essential modulator)) domain in NEMO forms a coiled coil, which binds two linear diubiquitins[7,8]. Extensive contacts are formed with both ubiquitin moieties, thus conveying specificity for linear ubiquitin chains. **d** | The structure of the single-helix, double-sided ubiquitin-interacting motif (UIM) of hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) bound to two ubiquitins reveals how it can target two ubiquitin molecules with equal affinity (PDB indentifier 2D3G)[23]. The ubiquitin molecules interact on opposite sides of the UIM and with a similar binding mode.

Diversity of degradation signals in the ubiquitin–proteasome system

Tommer Ravid and Mark Hochstrasser*[1]

Abstract | The ubiquitin–proteasome system degrades an enormous variety of proteins that contain specific degradation signals, or 'degrons'. Besides the degradation of regulatory proteins, almost every protein suffers from sporadic biosynthetic errors or misfolding. Such aberrant proteins can be recognized and rapidly degraded by cells. Structural and functional data on a handful of degrons allow several generalizations regarding their mechanism of action. We focus on different strategies of degron recognition by the ubiquitin system, and contrast regulatory degrons that are subject to signalling-dependent modification with those that are controlled by protein folding or assembly, as frequently occurs during protein quality control.

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A fundamental question regarding intracellular proteolysis is: how are specific proteins recognized by the proteolytic machinery such that they are degraded only under specific conditions with highly characteristic degradation rates? Early work suggested that global structural features determine the metabolic stability of

Segnale nella proteina da degradare: “degron”

Unfolded protein response:
unfolded, damaged proteins → expose degrons that otherwise are inside

1. The N-end rule pathway
2. E3α is the enzyme recognizin the N-terminus (RING-domain)

E3α, the mammalian genome encodes at least five other UBR box-containing proteins with specific signatures that are unique to E3 ubiquitin ligases[30]. Several of these puta-

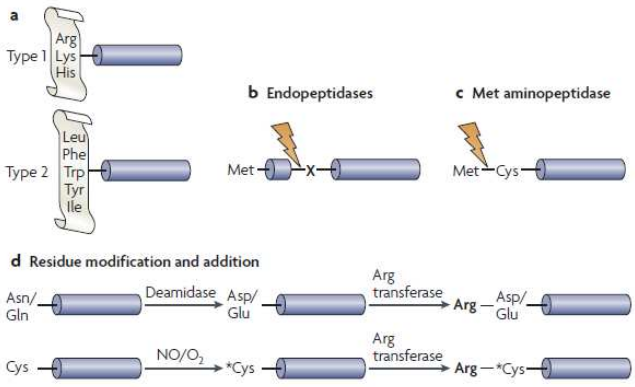

Figure 1 | **Mechanisms for the activation of N-end rule pathway substrates. a** | Type 1 (basic) and type 2 (hydrophobic) destabilizing residues of the N-end rule pathway are shown. **b** | Cleavage by endopeptidases (indicated by a lightning bolt) can lead to the positioning of a destabilizing residue (X) at the N terminus of the truncated protein. **c** | Cleavage between Met and Cys by Met aminopeptidase can lead to protein destabilization if followed by the addition of Arg, a type 1 destabilizing residue. **d** | Modification of Asn to Asp (or Gln to Glu) by specific deamidases can lead to the addition of Arg by arginyltransferase (upper panel). Oxidation (marked by an asterisk) of the N-terminal Cys residue can similarly lead to protein arginylation and degradation (lower panel).

Phosphodegrons

Phosphodegron recognition by SCF^{Cdc4}

F-box proteins (FBPs) are the substrate-specificity subunits of the multisubunit SCF family of E3 ligases, which contains S-phase-kinase-associated protein-1 (SKP1), cullin-1 (CUL1) and FBPs (BOX 3). Genetic and

The best-characterized phosphodegrons are those involved in the ordered elimination of specific cyclins and cyclin-dependent kinase (CDK) inhibitors by the ubiquitin system (see REFS 41,42 for reviews). In yeast,

dependent kinase-1; Cdk1) in late G1 phase. The primary function of Cln–Cdc28 kinase is to phosphorylate substrate inhibitor of Cdk1 (Sic1), an inhibitor of cyclin B-regulated kinase, thereby targeting Sic1 for degradation and enabling entry into S phase[43]. Phosphorylated Sic1 is specifically recognized by the FBP Cdc4, which is part of the SCF^{Cdc4} ubiquitin ligase. In the mammalian cell cycle, similar SCF complexes target phosphorylated forms of cyclin E and the CDK inhibitor p27^{KIP1} (REFS 44,45).

An oxygen-dependent degron

Another interesting example of protein ubiquitylation that is regulated by signal-dependent post-translational substrate modification occurs through hypoxia-inducible factor-1 (HIF-1), a heterodimeric transcriptional complex that mediates the transcriptional response to oxygen availability[50]. Under hypoxic conditions, HIF-1 activates the transcription of genes that are involved in the adaptation of cells to low oxygen tension, such as those that encode vascular endothelial growth factor and erythropoietin, which are important for the formation of new blood vessels and red blood cells. The HIF-1 complex is stable under hypoxia, but the HIF-1α subunit is rapidly degraded by the proteasome under normoxic conditions. This proteolytic regulation depends on a distinct cullin–RING ubiquitin ligase that is composed of von Hippel-Lindau protein (VHL), elongins B and C, the cullin CUL2A and RING-box-1 (RBX1) (REFS 51,52). VHL is the substrate-recognition subunit of the complex and binds to HIF-1α through an oxygen-dependent degron (ODD)[53,54]. In well-oxygenated cells, a HIF-1α-specific prolyl hydroxylase uses molecular oxygen to hydroxylate one or two specific prolyl residues[54,55].

Summary

•Ubiquitin is an intracellular signalling molecule that is conjugated to various proteins. Ubiquitin 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 through 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 development of disease, such as inflammation and cancer. The new structure-based insights provide strategies for the design of new approaches that can therapeutically target ubiquitin–UBD interaction surfaces.action surfa

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Molecular Cell
Review

A Role for Ubiquitin in Selective Autophagy

Vladimir Kirkin,[1,3,*] David G. McEwan,[1] Ivana Novak,[2] and Ivan Dikic[1,2,*]
[1]Institute of Biochemistry II and Cluster of Excellence Macromolecular Complexes, Goethe University, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany
[2]Mediterranean Institute for Life Sciences, Mestrovicevo setaliste bb, 21000 Split, Croatia
[3]Present address: Merck KGaA, Frankfurter Str. 250, D-64293 Darmstadt, Germany
*Correspondence: vladimir.chirchin@merck.de (V.K.), ivan.dikic@biochem2.de (I.D.)
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Ubiquitination is the hallmark of protein degradation by the 26S proteasome. However, the proteasome is limited in its capacity to degrade oligomeric and aggregated proteins. Removal of harmful protein aggregates is mediated by autophagy, a mechanism by which the cell sequesters cytosolic cargo and delivers it for degradation by the lysosome. Identification of autophagy receptors, such as p62/SQSTM1 and NBR1, which simultaneously bind both ubiquitin and autophagy-specific ubiquitin-like modifiers, LC3/GABARAP, has provided a molecular link between ubiquitination and autophagy. This review explores the hypothesis that ubiquitin represents a selective degradation signal suitable for targeting various types of cargo, ranging from protein aggregates to membrane-bound organelles and microbes.

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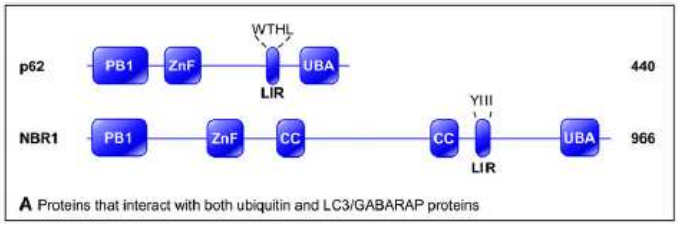
tion in lysosomes (Welchman et al., 2005). Recent experimental data have provided evidence for the involvement of Ub in yet another fundamental lysosome-dependent degradation system, autophagy. This catabolic pathway, capable of targeting individual proteins, larger macromolecular complexes, and complete organelles, is of great importance for cellular homeostasis and survival, while its deregulation has been linked to pathological conditions, such as neurodegeneration and cancer (Ohsumi, 2001; Xie and Klionsky, 2007; Levine and Kroemer, 2008).



A Proteins that interact with both ubiquitin and LC3/GABARAP proteins

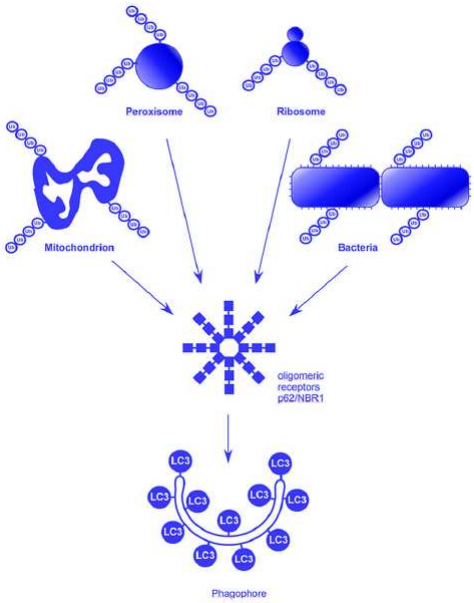


Figure 4. Proposed Role of Ubiquitination and Autophagy Receptors in Mitophagy, Pexophagy, Ribophagy and Xenophagy

Conjugation of monoUb (data not shown) or Ub chains (depicted) to the ribosomal proteins or those residing in the limiting membranes of organelles, such as mitochondria and peroxisomes, may constitute a signal that directs targeted autophagosome formation. Intracellular bacteria are also associated with Ub; however, the nature of this association is less clear. p62 and NBR1 form oligomers and bind both Ub and ATG8/LC3 on the phagophore and, thereby, might mechanistically link ubiquitination to selective autophagy.