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.

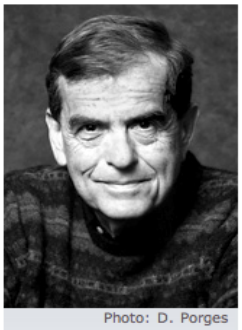
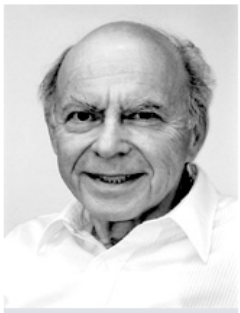


Photo: D. Porges

Aaron Ciechanover

Photo: D. Porges

Avram Hershko



Irwin Rose

1

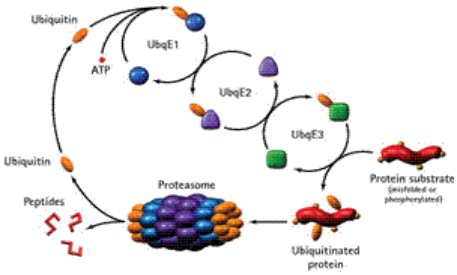
Ubiquitylation is among the most widely used protein modifications involved in regulating cellular signalling and homeostasis.

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.

2

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.



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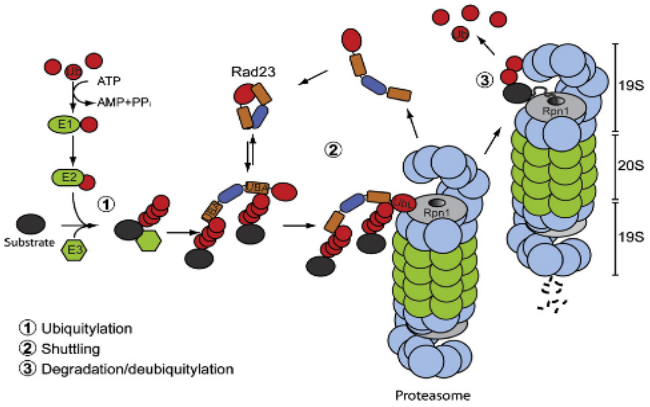

The role of Rad23 in ubiquitin-dependent proteasomal degradation. The UPS can be divided in ubiquitylation (left panel) and proteasomal degradation (right panel). Proteins harboring a degradation signal are ubiquitylated by an enzymatic cascade consisting of a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating 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

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

5

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

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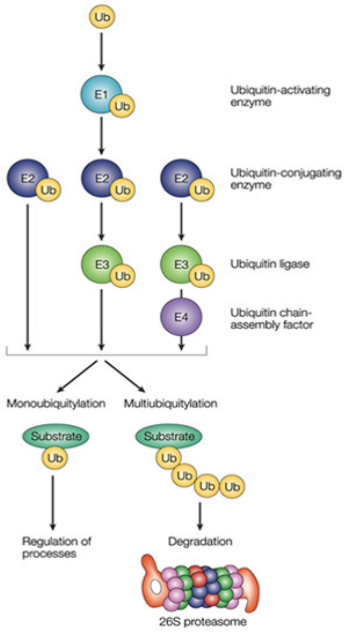
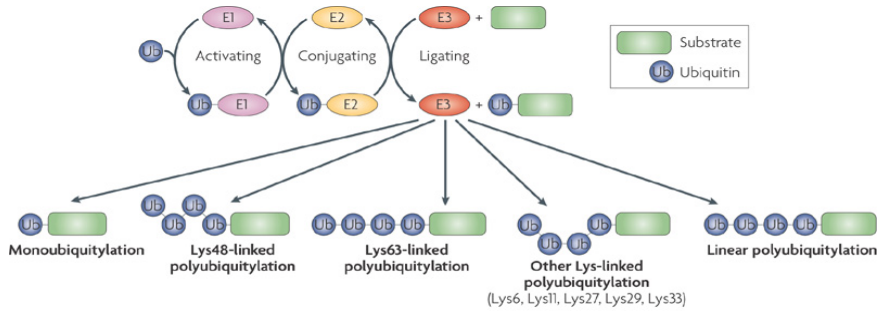
The ubiquitin pathway.

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
Veronika Jesenberger and Stefan Jentsch
Nature Reviews Molecular Cell Biology **3**, 112-121 (February 2002)

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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Nature Reviews Molecular Cell Biology **10**, 659-671 (October 2009)

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.

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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.

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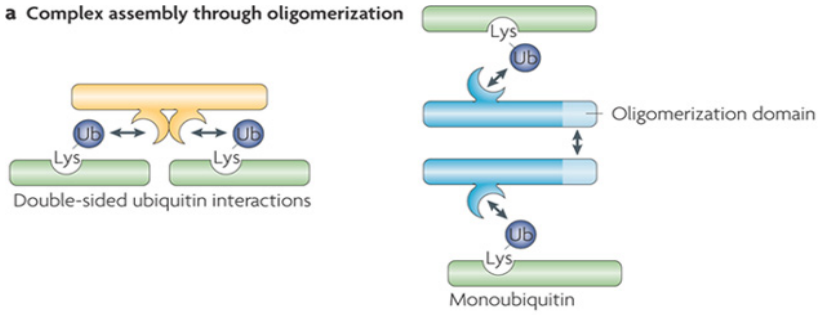
Specialized sets of ubiquitin-binding domains (UBDs) can read these post-translational modifications and mediate different outputs depending on the protein in which they are embedded.



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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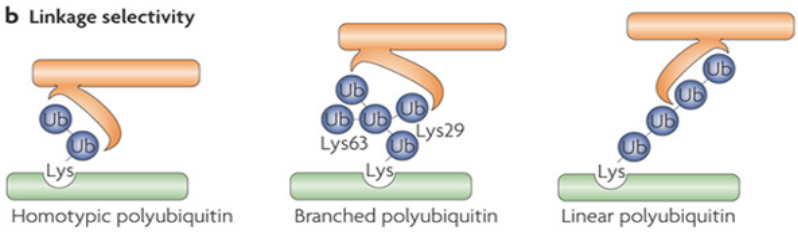
Nature Reviews Molecular Cell Biology **10**, 659-671 (October 2009)

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



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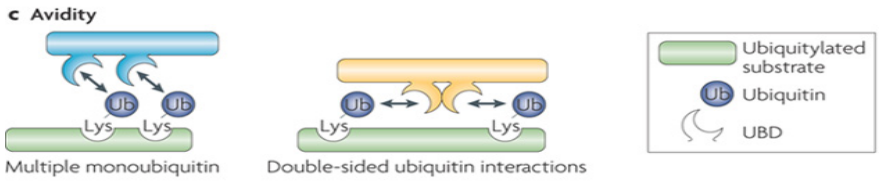

c | The presence of two or more UBDs in a protein or the attachment of multiple ubiquitin moieties on the same substrate can increase the avidity of and promote ubiquitin–UBD interactions, despite the low affinities of the individual interactions. This phenomenon might be important to filter noise coming 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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Nature Reviews Molecular Cell Biology **10**, 659-671 (October 2009)

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.

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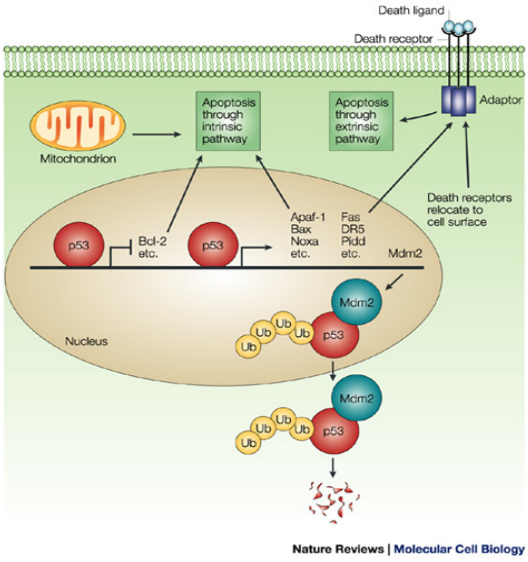
p53 and apoptosis

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.



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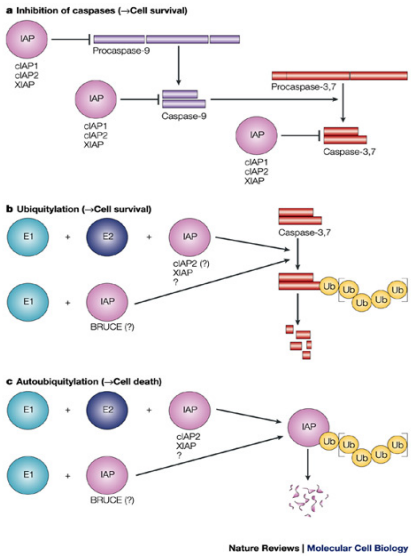
The multiple roles of IAPs.

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.



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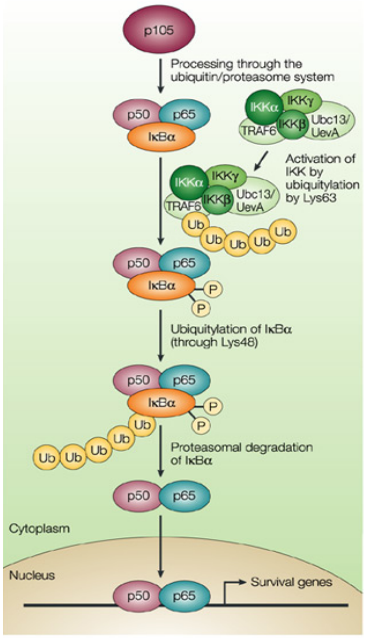
Control of NF-B activity by the ubiquitin/proteasome system.

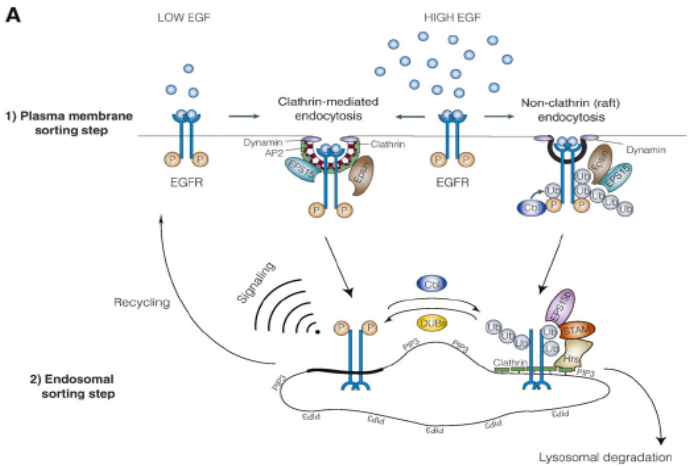
The most classical form of nuclear factor-B (NF-B) is a heterodimer of p50 and p65. The precursor form of p50, p105, is processed in a ubiquitin/proteasome-dependent manner to its mature form. p50 is present in the cytoplasm as a dimer with p65, and associated with an inhibitor of NF-B such as IB. By binding to NF-B, IB masks its nuclear localization signal, thereby preventing nuclear uptake. Following stimulation of cells by various agonists, IB is rapidly phosphorylated by the IB 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 IB serve as an essential part of a specific recognition site for the ubiquitin ligase RSIB/-TrCP, and IB is rapidly ubiquitylated and degraded by the proteasome. Following IB degradation, NF-B 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.



Nature Reviews | Molecular Cell Biology

Multiple sorting steps control EGFR and TGFβR trafficking and signaling.

(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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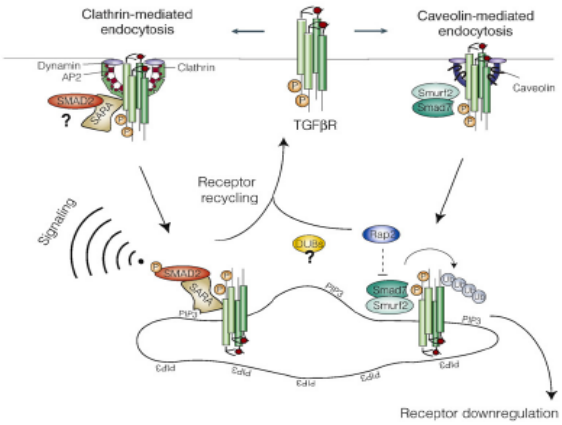
Multiple sorting steps control TGFβR trafficking and signaling.

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 communicatior 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.



19

Regulation of FOXO by monoubiquitination and deubiquitination.

a

(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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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

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DOI 10.1016/j.molcel.2009.04.026

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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Autophagy

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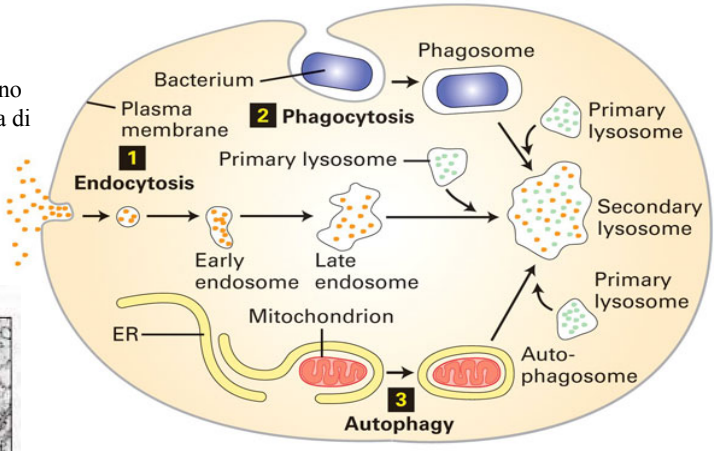
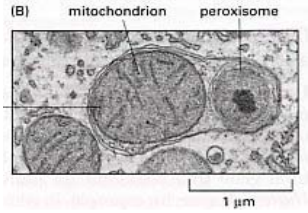
•Funzioni del REL: autofagia

Per autofagia si intende la degradazione di componenti della cellula stessa, siano essi macromolecole o organelli. In questo caso questi vengono circondati da vescicole derivanti dalla membrana del reticolo endoplasmatico, portando alla formazione del cosiddetto autofagosoma. Successivamente questo si fonde col lisosoma che è quindi libero di riversarvi il proprio contenuto.

Gli autofagosoma si distinguono dagli fagosoma per la presenza di una doppia membrana





Autofagosoma circondato da una doppia membrana e contenente un mitocondrio e un perossisoma.

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Autophagy

Autophagy is a **lysosomal degradation pathway for cytoplasmic material**. In mammalian cells autophagy is an important survival mechanism during short-term starvation. By degrading some non-essential components cells get nutrients for energy production and vital biosynthetic reactions. Autophagy also contributes to growth regulation and longevity. In addition, autophagy plays a role in innate immunity against viral infection and intracellular bacteria, as well as in the processing of viral antigens. Defective autophagy has been connected to many human diseases including cancer, myopathies, Alzheimer's disease, and Huntington's disease.

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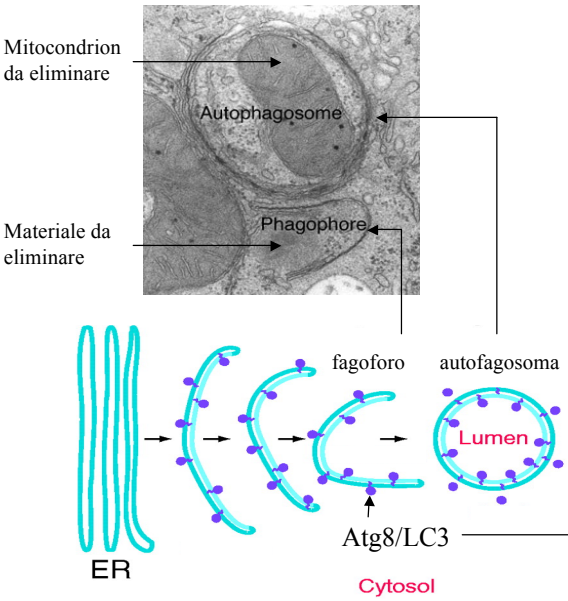
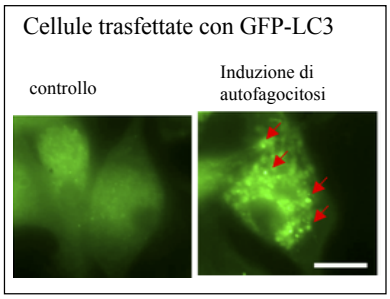
Formazione dell'autofagosoma:

Emissione di un prolungamento della membrana del RE e avvolgimento di materiale intracellulare da eliminare (organelli oppure materiale citoplasmatico compresi i ribosomi ad esempio).





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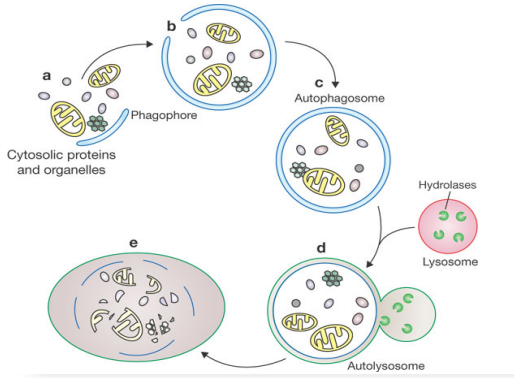
Fusione dell'autofagosoma con endosomi e/o lisosomi: formazione di anfisoma/autolisosoma: degradazione della membrana interna dell'autofagosoma e del contenuto

Schematic depiction of autophagy.
(a, b) Cytosolic material is sequestered by an expanding membrane sac, the phagophore, (c) resulting in the formation of a double-membrane vesicle, an autophagosome; (d) the outer membrane of the autophagosome subsequently fuses with a lysosome, exposing the inner single membrane of the autophagosome to lysosomal hydrolases; (d) the cargo-containing membrane compartment is then lysed, and the contents are degraded.



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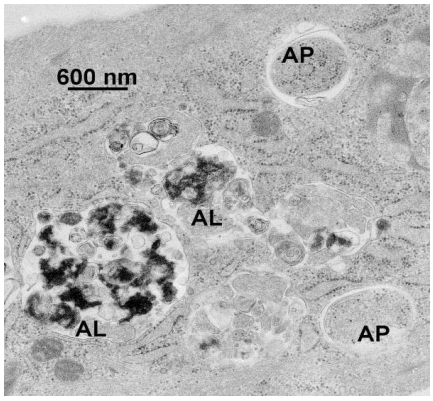

TEM demonstrating the ultrastructure of autophagosomes and amphisomes/autolysosomes in a mouse fibroblast. Early autophagosomes (AP) contain morphologically intact cytoplasm. Degradative amphisomes/autolysosomes (AL) contain partially degraded cytoplasmic material, above all remnants of ribosomes, which form electron dense amorphous aggregates.

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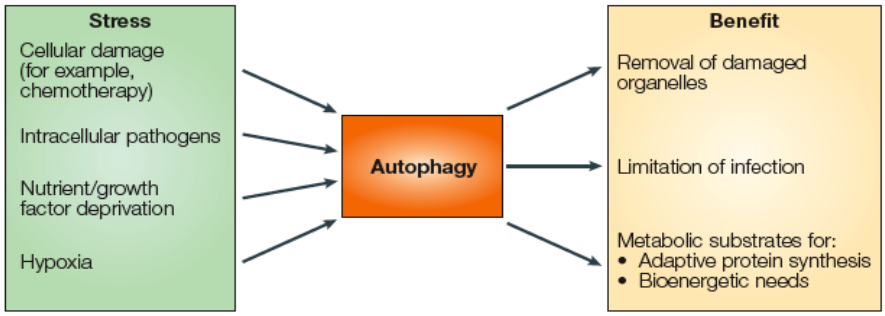

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