

Ubiquitin-mediated proteolysis: biological regulation via destruction

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Summary

The ubiquitin proteolytic system plays an important role in a broad array of basic cellular processes. Among these are regulation of cell cycle, modulation of the immune and inflammatory responses, control of signal transduction pathways, development and differentiation. These complex processes are controlled via specific degradation of a single or a subset of proteins. Degradation of a protein by the ubiquitin system involves two successive steps, conjugation of multiple moieties of ubiquitin and degradation of the tagged protein by the 26S proteasome. An important question concerns the identity of the mechanisms that underlie the high degree of specificity of the system. Substrate recognition is governed by a large family ubiquitin ligases that recognize the substrates, bind them and catalyze/facilitate their interaction with ubiquitin. *BioEssays* 22:442–451, 2000. © 2000 John Wiley & Sons, Inc.

Introduction

That modification by Ubiquitin provides a proteolytic signal was discovered during biochemical fractionation-reconstitution studies of rabbit reticulocyte lysates while studying ATP-dependent selective degradation of abnormal/misfolded proteins (reviewed in Ref. 1). Numerous molecular, biochemical, cellular, genetic and clinical studies have since unraveled the major role that ubiquitin-mediated proteolysis

plays in a broad array of basic cellular processes. Among these are regulation of the cell cycle, differentiation and development, the cellular response to extracellular effectors and stress, modulation of cell surface receptors and ion channels, DNA repair, regulation of the immune and inflammatory responses and biogenesis of organelles. Considering these numerous processes, it is not surprising that the system has been implicated in the pathogenesis of many diseases. In most cases, modification by ubiquitin targets the substrate for degradation by the 26S proteasome but, in certain cases, modification leads to targeting to the lysosome/vacuole. In contrast, the more recently discovered modification by ubiquitin-like proteins serves non-proteolytic functions such as routing of cellular proteins to their subcellular compartments. The list of cellular proteins targeted by ubiquitin is growing rapidly. Among them are cell cycle regulators, tumor suppressors and growth modulators, transcriptional activators and their inhibitors, cell surface receptors and endoplasmic reticulum proteins. Mutant proteins or otherwise damaged proteins are recognized specifically and, unlike their normal counterparts, removed rapidly.

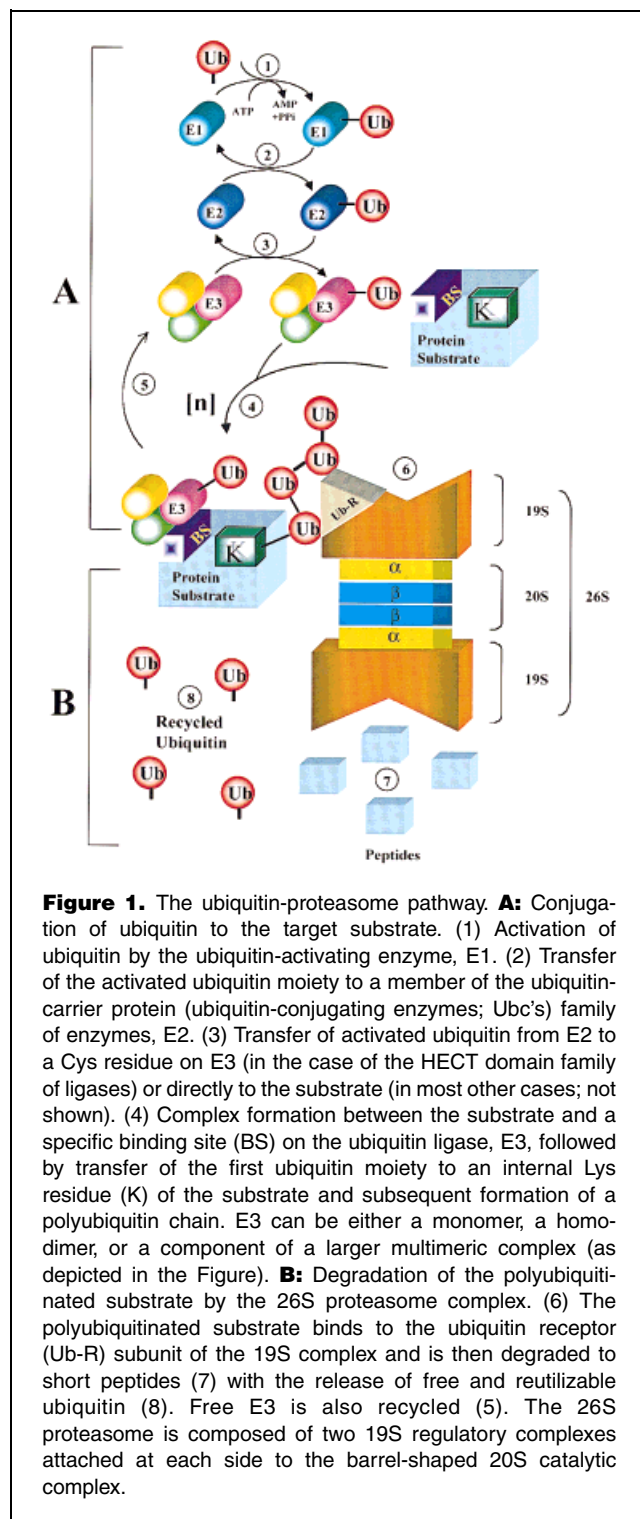
Degradation of a protein via the ubiquitin-proteasome pathway involves two successive steps: (1) covalent attachment of multiple ubiquitin molecules to the substrate; and (2) degradation of the tagged protein by the 26S proteasome and recycling of ubiquitin via the activity of ubiquitin C-terminal hydrolases (isopeptidases; see Fig. 1 for a scheme of the ubiquitin pathway). For recent reviews, see Refs 2–10. Conjugation of ubiquitin proceeds via a three step mechanism. Initially, the ubiquitin-activating enzyme, E1, activates the C-terminal Gly of ubiquitin to a high energy thiol ester with an internal E1 Cys residue. One of several E2 enzymes (ubiquitin-carrier proteins or ubiquitin-conjugating enzymes, Ubc's) transfers the activated ubiquitin, via an E2 ubiquitin thiol ester intermediate, to the substrate that is specifically bound to a member of the ubiquitin-protein ligase family, E3. Transfer can be either directly to the substrate, or via an additional E3 ubiquitin thiol ester intermediate. E3s facilitates/catalyzes covalent attachment of ubiquitin to the substrate. The first moiety is transferred to an ϵ -NH₂ group of internal Lys residue or to the α -NH₂ group⁽¹¹⁾ of the substrate to generate an isopeptide or a linear peptide bond,

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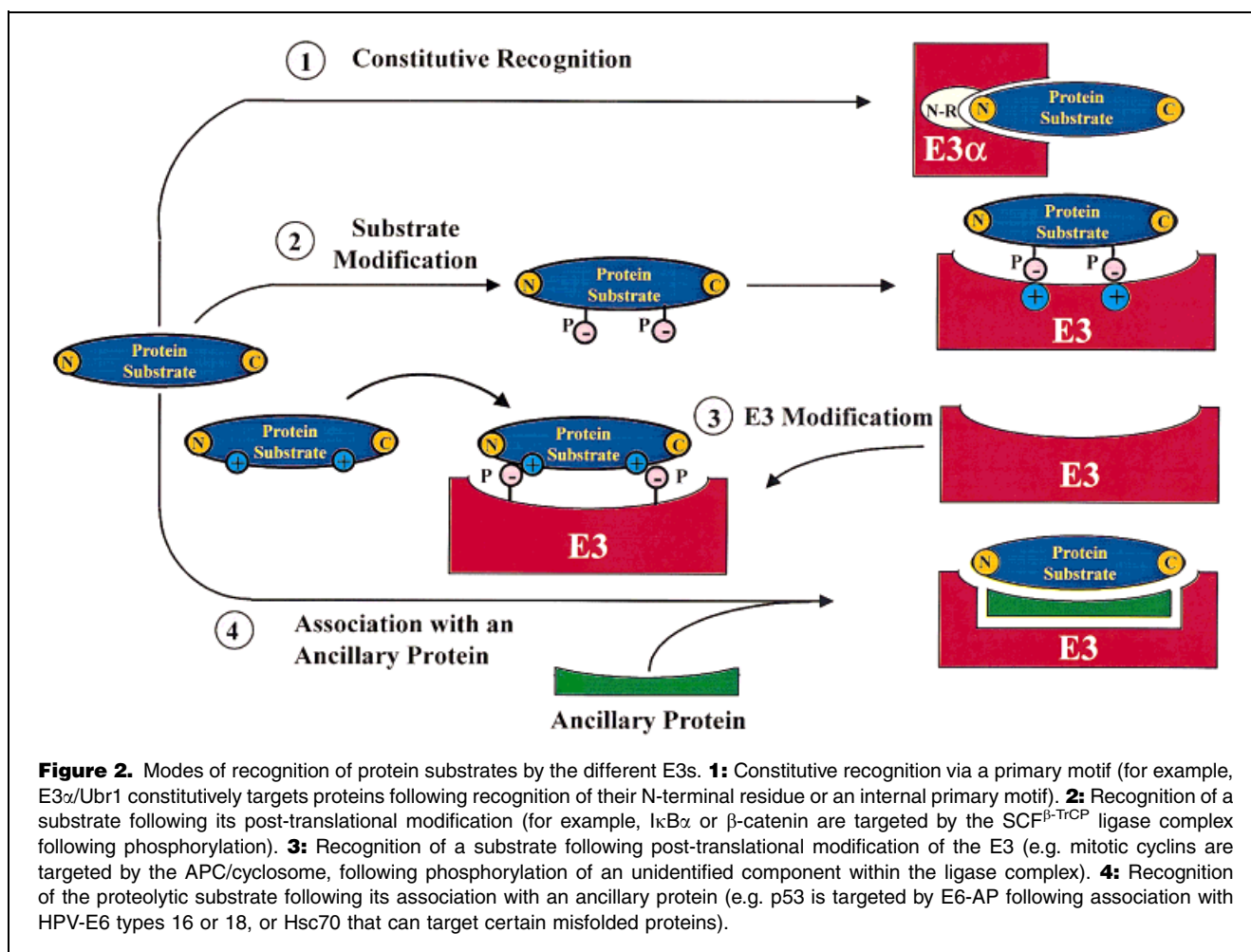


respectively. In successive reactions, a polyubiquitin chain is synthesized by transfer of additional activated ubiquitin moieties to an internal Lys residue of the previously conjugated ubiquitin molecule. While commonly, Lys₄₈ of ubiquitin is utilized for cross-linking, linkages to Lys₆₃⁽¹²⁾ or Lys₂₉⁽¹³⁾ have also been described, though they may have distinct functions. The chain serves, most probably, as a recognition marker for the protease.

The structure of the ubiquitin system is hierarchical: a single E1 species activates all the ubiquitin required for all modifications and transfers ubiquitin to several isoforms of E2. Each E2 isoform is able to act with either one or several E3 proteins. A limited number of E3 enzymes have been described thus far, but it appears that this is a large and rapidly growing family of proteins. E4 has been described recently and is involved in polyubiquitin chain elongation. Its activity, however, appears to be restricted to a limited subset of substrates.⁽¹⁴⁾

Although the components of the ubiquitin system have been localized to the cytosol and nucleus, targets of the system are known to include membrane-anchored and even ER luminal proteins. These proteins are 'retro-transported' to the cytosol, ubiquitinated, and degraded by the proteasome (reviewed in Refs. 15,16).

An important problem involves the mechanisms that underlie the high specificity of the system. Why are some proteins extremely stable while others are short-lived? And, why are some proteins degraded at a particular time during the cell cycle, or only following specific extracellular stimuli, while they are stable under most other conditions? Specificity is imparted by two distinct groups of proteins. Within the ubiquitin system, protein substrates must be recognized and bind to an E3 enzyme prior to their modification. Recognition is mediated by specific structural motifs within the substrate. Some of these motifs are encoded within the protein itself and the proteins that harbor them are degraded constitutively. Stability of other proteins depends on their state of oligomerization, post-translational modification, such as phosphorylation, or association with ancillary proteins such as molecular chaperones, that act as recognition elements in *trans*. Some transcription factors must dissociate from the specific DNA to which they bind in order to be recognized. In other cases, it is the E3 enzyme or a subunit of the E3 complex that must be modified in order to be active (see Fig. 2 for the different modes of recognition). Thus, in addition to the central role of E3 proteins in the recognition process, the modifying enzymes, ancillary proteins, and specific DNA binding sites also play an important role. It is rare that a single protein is targeted by a specific E3 ligase, and in most cases, an E3 recognizes a subset of proteins that contain similar structural motifs. Some proteins are recognized by two different E3 enzymes, via distinct recognition motifs.



Enzymes of the ubiquitin system

E1 and E2s

A single E1 catalyzes all ubiquitination reactions, and inactivation of this gene is lethal. The yeast genome encodes 11 E2 enzymes denoted Ubc1–8, 10, 11, 13; Ubc9 and Ubc12, although members of the Ubc family, are involved in conjugation of ubiquitin-like proteins. E1 and E2 can catalyze transfer of a single ubiquitin moiety to target proteins *in vitro*. The role of mono-ubiquitin adducts is not known as they are not recognized by the 26S proteasome and catalysis of polyubiquitination that renders the substrate susceptible for degradation requires E3.

Ubiquitin ligases, E3s

It is the E3 ligases that provide the high specificity of the system, although these are the least defined components of the pathway. The ligase is a protein or a protein complex that binds both the E2 and the substrate. Interaction with the substrate is either direct or via an ancillary protein. Most often,

the E3 serves as a scaffold protein that brings together the E2 and the substrate. In some cases, the activated ubiquitin is transferred from E2 to an internal Cys residue on E3 prior to its conjugation to the target. Here, the E3 has a catalytic role. E3 ligases can be subdivided into at least six subtypes with regard to structure and/or class of signals they recognize.

(1) E3 α (Ubr1 in yeast; reviewed in Ref. 17) recognizes 'destabilizing' N-terminal residues ('N-end rule' substrates). The physiological significance of the 'N-end rule' pathway is not clear, since it is not essential in yeast, and only a few natural substrates traverse it. E3 α has two defined binding sites, for basic and bulky hydrophobic N-terminal residues. Importantly, it also recognizes proteins via internal, downstream signals. E3 β is a ligase related to E3 α that targets proteins with small uncharged N-terminal residues.⁽¹⁸⁾

(2) HECT-domain proteins (homologous to E6-AP carboxyl terminus) contain a 350 residue domain homologous to the C-terminal domain of the prototypical member of the family, E6-AP (E6-associate protein).⁽¹⁹⁾ This domain contains a

conserved Cys residue to which the activated ubiquitin moiety is transferred from E2.⁽²⁰⁾ E6-AP targets p53 for rapid degradation in the presence of the human papillomavirus (HPV) oncoprotein E6.⁽²¹⁾ It also targets native cellular proteins, such as Blk (a member of the Src family of kinases) in the absence of E6.⁽²²⁾ Other HECT-domain E3 ligases target the kidney epithelial Na⁺ channel,⁽²³⁾ the yeast uracil and amino acid permeases,⁽²⁴⁾ and SMADs specific to the bone morphogenetic protein (BMP) pathway.⁽²⁵⁾

(3) The anaphase promoting complex (APC)⁽²⁶⁾ or cyclosome⁽²⁷⁾ consists of at least eight subunits⁽²⁸⁾ and targets mitotic substrates. While several subunits of the APC contain tetratricopeptide (TPR) repeats that are involved in protein-protein interactions, the identity of the E3 subunit has remained elusive. At least two, sub-stoichiometric, ancillary factors have been identified that regulate its activity. The yeast proteins are Cdc20 and Hct1/Cdh1,⁽²⁹⁾ whereas the human homologs are p55Cdc/hCdc20 and hCdh1.⁽³⁰⁾ These proteins confer substrate specificity to APC. For example, Cdc20-APC is active at the beginning of anaphase, when it degrades the anaphase entry inhibitor Pds1, whereas Hct1/Cdh1 is required for the degradation of cyclin B at the end of mitosis. Both proteins are regulated. Failure of spindle assembly leads to binding of the Mad1-3 checkpoint protein to Cdc20, which results in inhibition of mitosis.⁽³¹⁾ CDK/Cyclin B phosphorylates Hct1/Cdh1 which inhibits its binding to APC.⁽³²⁾ APC itself is a target of an activating phosphorylation by cyclin B/Cdk1.⁽³³⁾ The best-characterized APC substrates are the A- and B-type cyclins. These, and other, APC substrates contain a degradation signal designated the 'destruction box' which has the following consensus sequence, **R-A/T-A-L-G-X-I/V-G/T-N** (indispensable residues are in bold; reviewed in Ref. 8). The role of this sequence is, however, still obscure.

(4) The SCF complexes (Skp1, yeast Cdc53, or mammalian Cullin, and F-box protein) act with the E2 Cdc34/Ubc3,⁽⁹⁾ and possibly with members of the UbcH5.⁽³⁴⁾ Recently, a fourth, Rbx1,⁽³⁵⁾ or Roc1,⁽³⁶⁾ and also, potentially a fifth, Sgt1⁽³⁷⁾ component of this complex have been identified. The catalytic complex may have the following hexameric structure: E2 · Rbx1/Roc1 · Cdc53/Cullin-1 · Skp1 · F-Box protein · Protein substrate. The role of Sgt1 is not yet clear. The Rbx1/Roc1, Skp1, and Cdc53/Cullin-1 subunits are probably common to all SCF complexes. The F-box protein which binds specific substrates is the variable component, and the different complexes are designated according to the F-box component (e.g. SCF^{Cdc4}, SCF^{β-TrCP}, SCF^{Skp2}). SCF^{β-TrCP} targets phosphorylated IκBα,⁽³⁸⁾ β-catenin,⁽³⁹⁾ and HIV-1-Vpu⁽⁴⁰⁾ (binding of Vpu forms a ternary complex, Vpu-CD4-SCF, with subsequent degradation of the CD4 receptor). The signal recognized by SCF^{β-TrCP} in all three substrates is DS(P)GΨXS(P), though other potential substrates, which are yet to be identified, could contain a different signals. SCF^{Skp2}

targets E2F-1⁽⁴¹⁾ and p27^{Kip1}.⁽⁴²⁾ Although p27^{Kip1} must be phosphorylated on Thr₁₈₇ in order to be recognized, it is not clear whether targeting of E2F-1 also requires phosphorylation.

(5) Several ring finger proteins also appear to serve as ubiquitin ligases.⁽⁴³⁾ For example, the c-Cbl proto-oncoprotein stimulates CSF-1 receptor ubiquitination and endocytosis.⁽⁴⁴⁾ Similarly, it targets the EGF and PDGF receptors.^(45,46) The ring finger domain plays an important role in the function of the ligase, possibly in the formation of the polyubiquitin chain.⁽⁴⁷⁾

(6) The von Hippel-Lindau tumor suppressor protein (pVHL) is part of a complex that includes Elongins B and C, Cullin-2, and Rbx1/Roc1 and is similar to SCF complexes. This complex can catalyze polyubiquitination,^(48,49) and the recent discovery that it targets hypoxia-inducible factors (HIFs) for degradation⁽⁵⁰⁾ raises the possibility that it serves as their ligase.

The 26S proteasome

The proteasome is a large, 26S, multicatalytic protease that degrades polyubiquitinated proteins to small peptides (reviewed recently in Refs. 5,6). It is composed of two sub-complexes, a core catalytic 20S particle and a regulatory 19S particle. The 20S complex is barrel-shaped and consists of four stacked rings, two identical outer α rings and two identical inner β rings. The α and β rings are composed each of seven distinct subunits, giving the complex the general structure of α₁₋₇β₁₋₇β₁₋₇α₁₋₇. The catalytic sites are localized in some of the β subunits. Electron microscopy analysis shows that each extremity of the 20S complex is capped by a 19S particle. The yeast 19S complex has been further resolved into two sub-complexes, the 'base', which consists of 6 ATPases of the AAA family and three additional proteins, and an 8-subunit 'lid' that is homologous to cop-signalosome complexes.⁽⁵¹⁾ The function of the signalosomes is not known, and, therefore, the significance of the homology is also an enigma.

One function of the 19S complex is to recognize ubiquitinated proteins and other substrates of the proteasome. A ubiquitin-binding subunit, Rpn10/Mcb1, has been identified in the 19S particle. Since this subunit is not essential in yeast, however,⁽⁵²⁾ there must be additional ubiquitin-binding component(s) in the 19S complex. A second function of the 19S complex, probably conferred by the ATPase subunits, is to create a 'gate' in the α ring through which substrates can be inserted into the proteolytic chamber: the yeast 20S particle seems to be occluded at both ends by the protruding termini of the α subunits.⁽⁵³⁾ Finally, it is assumed that the 19S particle unfolds the polypeptide chain so it can be inserted into the proteolytic chamber. Interestingly, the 'base' sub-complex exhibits a chaperone-like activity in refolding denatured proteins.⁽⁵⁴⁾ Thus, during proteolysis it may act in an 'opposite' direction.

Deubiquitinating enzymes

The cell contains many deubiquitinating enzymes that cleave ubiquitin molecules conjugated via either isopeptide or linear peptide bonds. These processes are essential for the maturation of newly synthesized ubiquitin molecules which are often translated as a linear, 'head' to 'tail', polyubiquitin molecules, for the release of some ribosomal proteins that are synthesized fused at their N-terminal residue to a ubiquitin moiety that targets them to the ribosome, and for the recycling of ubiquitin molecules after degradation of the substrate (reviewed in Ref. 55). Inactivation of the major deubiquitinating enzymes results in inhibition of ubiquitin-mediated proteolysis. This is probably a consequence of two effects: depletion of the cellular free ubiquitin pool, and saturation of the proteasome with cleaved, substrate-free, polyubiquitin chains. Deubiquitinating enzymes can also accelerate proteolysis by 'trimming' polyubiquitin chains to a length that is most efficiently recognized by the 26S proteasome. Deubiquitination may also serve a 'correction' function by releasing ubiquitin chains from substrates that have been spuriously conjugated.

Deubiquitination also has specific regulatory roles. For example, specific deubiquitinating enzymes affect transcriptional silencing in *S. cerevisiae*⁽⁵⁶⁾ and eye development in *Drosophila*.⁽⁵⁷⁾ DUB(deubiquitinating)-1 and DUB-2 are induced by cytokines, and high-level expression of DUB-1 leads to cell-cycle arrest.⁽⁵⁸⁾ An intragenic deletion in the gene encoding the ubiquitin-carboxyl-terminal hydrolase (UCH) isozyme Uch-L1 results in the gad (gracile axonal dystrophy) syndrome in mice.⁽⁵⁹⁾ This is characterized by sensory and motor ataxia and neurodegeneration accompanied by the accumulation of ubiquitin conjugates (see also below for mutations in human UCH-L1). In all these cases however, the target proteins remain, as yet, unknown.

Sites of intracellular degradation

Ubiquitin-mediated degradation of cytosolic and membrane proteins occurs in the cytosol and on the cytosolic face of the ER membranes (reviewed in Refs. 15,16). Although components of the system have been localized to the nucleus, conjugation and degradation have not been demonstrated in this organelle. Leptomycin B, a drug that inhibits nuclear export, almost completely prevents Mdm2- and E6-AP/E6-dependent degradation of p53.⁽⁶⁰⁾ The degradation of p27^{Kip1} probably occurs also in the cytosol, following Jab1 (p38)-mediated nuclear export.⁽⁶¹⁾ The physiological significance of regulation via nuclear-cytoplasmic shuttling and of the physiological role of the components of the ubiquitin pathway in the nucleus are still mysterious.

'Alternative' pathways

As described above, the two 'arms' of the ubiquitin-proteasome pathway are polyubiquitination of the substrate

followed by its proteasome-mediated degradation. The two arms of the pathway can also function independently, however. For example, ornithine decarboxylase (ODC) is degraded by the proteasome without prior ubiquitination. It appears that antizyme, an inhibitor of ODC, mediates recognition of non-ubiquitinated ODC by the proteasome.⁽⁶²⁾ In other cases, ubiquitination of membrane receptors or transporters results in their internalization and degradation in the vacuole/lysosome (reviewed in Refs. 15,16,63). Unlike proteasomal degradation, which requires polyubiquitination, mono-ubiquitination appears to be sufficient for receptor internalization, at least in some cases. Furthermore, mono-ubiquitination can occur on Lys residues other than Lys₄₈, for example, the mono-ubiquitination required for endocytosis of certain membrane receptors can occur on Lys₆₃ (reviewed in Ref. 63). One exception to the rule that ubiquitination leads to protein degradation is the limited processing of p105, the precursor of the transcription factor NF- κ B. The C-terminal domain of the molecule is degraded specifically, leaving behind p50, the active subunit of the factor. Cleavage requires the appropriate positioning of three domains, a Gly-rich region that acts as a processing 'stop' signal,⁽⁶⁴⁾ a ubiquitination/E3 anchoring domain,⁽⁶⁵⁾ and a C-terminal domain that is regulated by phosphorylation.⁽⁶⁶⁾

Regulation of ubiquitin-mediated proteolysis

General regulation

The ubiquitin pathway can potentially be regulated at the level of either ubiquitination or proteasome activity. It appears that the general components of the system, E1, E2s, and the proteasome, are constitutively active, or may be regulated to only a limited extent. This is, perhaps, to be expected, since these components are involved in the degradation of a multitude of substrates, and alteration in their activity will affect many substrates. Regulation of the general components have, however, been demonstrated in two cases. One is the up-regulation of the ubiquitin pathway to achieve bulk degradation of skeletal muscle proteins that occurs in different pathophysiological conditions such as fasting, cancer cachexia, severe sepsis, metabolic acidosis, or following denervation (reviewed in Ref. 67; see also below).⁽⁶⁷⁾ This occurs also during specific developmental processes, such as insect metamorphosis where massive breakdown of larval muscle tissue occurs prior to the development of the pupa and the mature butterfly.⁽⁶⁸⁾ The second example of a change in the general components of the system occurs following treatment with IFN- γ . This cytokine induces changes in the subunit composition of the 20S proteasomal complex. Consequently, the antigenic peptides that are generated following proteasomal degradation have higher affinity for the presenting MHC class I molecules and for the cytotoxic T-cell receptor (reviewed in Ref. 7).

Specific regulation

Degradation of specific substrates is regulated mostly at the level of ubiquitination. Regulation can be mediated via post-translational modification or a structural change of the substrate that render it susceptible to recognition by the E3 ligase, or via modulation of the activity of specific E2/E3 complexes. From studies thus far, it appears that the mode of regulation correlates with the class of E3. Ubiquitination by SCF complexes requires phosphorylation of the substrate, the APC activity is modulated by the presence of activators and by phosphorylation, and the activity of at least some HECT-domain proteins towards certain substrates may depend on ancillary proteins that facilitate recognition *in trans*.

Regulation by modification of the substrate: Phosphorylation of many substrates is required for their recognition by their E3s. Conversely, similar modification of many other proteins prevents this. Substrates that require prior phosphorylation include the yeast G1 cyclins, Cln2 and Cln3, the yeast cyclin-dependent kinase (CDK) inhibitors, Sic1 and Far1, the mammalian G1 regulators, cyclins D and E, the mammalian CDK inhibitor, p27^{Kip1}, the mammalian transcriptional regulators, I κ B α and β -catenin, and the viral protein HIV-1-Vpu. In all of these cases, the E3 was identified as a member of the SCF family of complexes (reviewed in Refs. 2–4,9). Proteolysis of I κ B α , β -catenin and binding to HIV-1 Vpu is mediated by SCF ^{β -TrCP} following specific phosphorylation of two serine residues that reside within a defined consensus sequence DS(P)G Ψ XS(P).^(38–40) The activity of the ligase towards the substrates is constitutive, and the regulatory step is the phosphorylation (in the case of I κ B α) or dephosphorylation (in the case of β -catenin). Unlike β -TrCP, no consensus recognition motif(s) have been defined for other F-box proteins. Sic1 and p27^{Kip1} are G1 CDK inhibitors, which must be degraded in order to allow entry into the S phase. Phosphorylation at several Thr and Ser residues in the N-terminal domain of Sic1 and phosphorylation of p27^{Kip1} at Thr₁₈₇ are required for their recognition by SCF^{Cdc4} and SCF^{Skp2}, respectively. Here too, the SCF complexes are constitutive, and phosphorylation of the substrates is the regulated step.

Interestingly, ubiquitination of an SCF substrate can also be modulated by the presence or absence of the specific F-box protein. E2F-1 and p27^{Kip1} are ubiquitinated by SCF^{Skp2}.^(41,42) Skp2 expression and activity are regulated during the cell-cycle, with a peak of expression during S phase.⁽⁶⁹⁾ Modulation of the expression of the F-box protein might confer an added level of regulation.

Degradation of the proto-oncogene c-mos by the ubiquitin pathway is inhibited by phosphorylation on Ser⁽⁷⁰⁾. Interestingly, activation of c-mos leads to phosphorylation and stabilization of c-fos, another substrate of the ubiquitin pathway.⁽⁷¹⁾ Another example is that of the anti-apoptotic protein Bcl-2. Dephosphorylation of Bcl-2 following apoptotic

stimuli renders it susceptible to degradation by the ubiquitin pathway.⁽⁷²⁾

Regulation by modulation of ubiquitination activity: Regulated degradation of specific classes of substrates could be achieved by modulation of the activity of the ubiquitination machinery. For example, it has been shown recently that degradation of mitotic regulators by the APC is regulated by different activators and inhibitors and by phosphorylation (Refs. 29–33 and see above).

Regulation by ancillary proteins: Several viral proteins exploit the ubiquitin system by targeting for degradation cellular substrates which may interfere with propagation of the virus. In some instances, the viral protein functions as a 'bridging' element between the E3 and the substrate, thus conferring recognition *in trans*. The prototype of such a protein is the high risk HPV oncoprotein E6 which interacts with an E6-AP HECT domain E3, and with the tumor suppressor protein p53. This interaction targets p53 for rapid degradation and, thus, most probably prevents stress signal-induced apoptosis and ensures further replication propagation of the virus (Ref. 21 and see above). In a different case, the Vpu protein of the HIV-1 virus is recognized by the F-box protein, β -TrCP.⁽⁴⁰⁾ Vpu also binds to the CD4 receptor in the ER of T cells infected by the virus. This leads to ubiquitination and subsequent degradation of CD4 by the SCF ^{β -TrCP} complex, thus enabling the virus to escape from immune surveillance.

Degradation of many misfolded/denatured proteins is mediated by formation of a complex with molecular chaperones such as Ydj1 in yeast⁽⁷³⁾ and Hsc70 in mammals.⁽⁷⁴⁾ If the chaperone is unable to refold a denatured substrate, despite several repeated cycles of association and dissociation, it presents the misfolded protein to the ligase for ubiquitination and subsequent degradation. This is the 'refold or degrade' function of the chaperone.

Regulation by masking of a degradation signal: The presence of either one of two transcription factors, MATa1 and MAT α 2, determines the mating type of haploid yeast cells. The diploid cell expresses both a1 and α 2 that form a heterodimer with distinct DNA-binding specificity. In haploid cells, the two factors are rapidly degraded by the ubiquitin system. Degradation of α 2 requires two degradation signals, Deg1 and Deg2. Strikingly, both a1 and α 2 are stabilized by heterodimerization.⁽⁷⁵⁾ For α 2 at least, it has been shown that residues required for interaction with a1 overlap with the Deg1 degradation signal and it is possible that binding of a1 interferes with the degradation of α 2 by masking the ubiquitin recognition signal. A similar mechanism is thought to protect the *Drosophila* homeobox protein Homothorax in cells expressing its binding partner Extradenticle.⁽⁷⁶⁾ An analogous observation involves the transcription factor MyoD which is protected from conjugation by binding to its cognate DNA sequence.⁽⁷⁷⁾

The ubiquitin system and pathogenesis of human diseases

As mentioned above, the ubiquitin system has been implicated, both directly and indirectly, in the pathogenesis of several important human diseases. Angelman syndrome is characterized by severe motor and mental retardation and is caused by mutations in the ubiquitin ligase E6-AP.⁽⁷⁸⁾ The substrate(s) that accumulates and is toxic to the developing brain cells has not been identified. Liddle's syndrome, is a severe form of hypertension that is due to a mutation in the PPxY motif of the kidney epithelial Na⁺ channel (ENaC). The mutant channel is unable to interact with the WW domain of its ubiquitin ligase Nedd4 and the consequent stabilization of the channel leads to increased reabsorption of Na⁺ and H₂O (Ref. 23 and see above). Targeting of the tumor suppressor protein p53 for degradation by the human papillomavirus oncoprotein E6 and the ubiquitin ligase E6-AP has been implicated in the pathogenesis of human uterine cervical carcinoma.^(21,79)

Less direct evidence implicates the ubiquitin system in the pathogenesis of many other diseases. The level of the cyclin-dependent kinase (CDK) inhibitor p27^{Kip1}, which acts as a negative growth regulator/tumor suppressor, is inversely correlated with the aggressiveness and severity of many types of malignancies, including colorectal, breast, and prostate carcinomas (reviewed in Ref. 80). Interestingly, the low level of p27 in the most aggressive tumors is due to specific activation of the ubiquitin system, as the p27 that is found in these tumors is of the WT species.

The ubiquitin system has also been implicated in the pathogenesis of several neurodegenerative diseases. A frameshift mutation in a 'hot spot' motif in the transcript of ubiquitin and β -amyloid precursor protein (β -APP) has been described in many patients with late onset Alzheimer's disease.⁽⁸¹⁾ A missense mutation in the gene encoding for the UCH-L1 has been described in a German family with Parkinson's disease.⁽⁸²⁾ Ubiquitin adducts have been described in many neurodegenerative diseases. Immunohistochemical analyses have revealed the existence of ubiquitinated neurofibrillary tangles, senile plaques, and paired helical filaments in Alzheimer's disease patients.⁽⁸³⁾ Similar analyses revealed ubiquitin-modified Huntingtin in Huntington's disease⁽⁸⁴⁾ and ubiquitinated Ataxin-1 in Spinocerebellar Ataxia type-1.⁽⁸⁵⁾ Interestingly, both Huntingtin and ataxins have an N-terminal poly-glutamine repeat that probably interferes with their normal catabolism. It is not clear whether these conjugates play a secondary pathogenetic role or whether they represent non-toxic scavenger products of the mutated abnormal/misfolded proteins that cannot be degraded by the 26S proteasome.

Microdeletions in chromosome 22q11 are the most common defects associated with cardiac and craniofacial

anomalies. Two syndromes, DiGeorge syndrome (DGS) and velo-cardio-facial syndrome (VCFS) have been attributed to deletions in this region. The *UFD1L* (*ubiquitin fusion degradation*) gene is deleted in most of the patients with 22q11 deletion.⁽⁸⁶⁾ The UFD pathway is involved in the degradation of model proteins to which ubiquitin is stably fused at the N-terminal residue.⁽⁸⁷⁾ While the native cellular substrates of this pathway have not been identified, they may include proteins to which the first ubiquitin moiety is fused to the N-terminal residue.⁽¹¹⁾ UFDL1 is an enzyme that functions in a post-ubiquitination step and can be, for example, an isopeptidase.

The *CF* (*Cystic Fibrosis*) gene encodes the CFTR (CF Transmembrane Conductance Regulator) which is a chloride channel. Normally, only a small fraction of the WT protein matures to the cell surface, whereas most of the protein is degraded from the ER by the ubiquitin system.⁽⁸⁸⁾ The most frequent mutation in CFTR is $\Delta F508$. Despite normal ion channel function, CFTR ^{$\Delta F508$} does not reach the cell surface, and is retained in the ER from which it is degraded. It is possible that the efficient degradation that results in complete lack of cell surface CFTR ^{$\Delta F508$} , contributes to the pathogenesis of the disease.

Activation of the ubiquitin pathway is also observed during severe muscle wasting that occurs in certain forms of sepsis, cachexia, renal insufficiency, and following denervation.⁽⁶⁷⁾ The signals involved in eliciting the accelerated degradation of muscle proteins have not been identified.

Two interesting examples illustrate how viruses exploit the ubiquitin system to escape immune surveillance, most likely resulting in persistence and/or exacerbation of the infection. The Epstein Barr Nuclear Antigen 1 (EBNA-1) protein persists in healthy carriers for life, and is the only viral protein detected in all EBV-associated malignancies. The persistence of EBNA-1 most probably contributes to some of the virus-related pathologies. Unlike EBNA-2-4, which are strong immunogens, EBNA-1 does not elicit a CTL response. A long C-terminal Gly-Ala repeat in EBNA-1 inhibits ubiquitin-mediated degradation and subsequent antigen presentation.⁽⁸⁹⁾ Interestingly, a Gly rich region in p105 prevents its degradation and serves as a 'stop' signal that is essential for its limited processing (Refs. 64, 65 and see above). A second example of how viruses utilize the ubiquitin pathways involves the human cytomegalovirus (CMV). This virus encodes two ER-resident proteins, US2 and US11 which bind to the MHC molecules in the ER and escort them to the translocation machinery. Following retrograde transport to the cytoplasm, they are ubiquitinated and degraded by the 26S' proteasome.⁽⁹⁰⁾ The virus-mediated destruction of the MHC molecules prevents presentation of antigenic viral peptides at the cell surface and, thus, enables the virus to evade the immune system.

Conclusions and future perspectives

The discovery of the ubiquitin pathway with its many substrates and functions, has revolutionized our conception of intracellular protein degradation. Unlike the initial image of an unregulated, non-specific terminal scavenger process, it has become clear that proteolysis of cellular proteins is a highly complex, temporally controlled, and tightly regulated, process that plays important roles in a broad array of basic cellular processes. Despite this progress, the unknown still exceeds our knowledge of the system, and the full range of the target proteins, as well as that of the different ligases, are yet to be revealed. Only a few targeting signals have been identified, and the mechanisms that underlie the regulation of the system are still largely unknown. While the system has been implicated in the pathogenesis of several diseases, the underlying mechanisms, as well as its potential involvement in many other diseases, are still an enigma. Deciphering the complete scope of the system and its modes of action will lead not only to better understanding of basic regulatory mechanisms, but also to the development of strategies and drugs that will specifically modulate the different processes.

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