The Ubiquitin-Proteasome System: Death of Proteins is Required for Life of Cells

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Between the 1960s and 1980s, much of life science research was focused on the study of nucleic acids and the translation of their encoded information into the synthesis of new proteins. In comparison, protein degradation was a neglected area, considered to be a non-specific, dead-end process. While it was known that proteins turn over, the large extent and high specificity of the process, whereby distinct proteins can have half-lives that range from a few minutes to several days, was not appreciated. However, beginning in 1978 and throughout the next decade, the discovery of the complex cascade of the ubiquitin proteasome system (UPS) dramatically changed the field. As a result, it is now clear that the degradation of cellular proteins is a highly complex, temporally controlled and tightly regulated process that plays major roles in a variety of basic cellular pathways during both cell life and cell death, and hence in both health and disease. With the multitude of protein substrates targeted and the myriad of processes involved, it is not surprising that aberrations in the UPS are implicated in the pathogenesis of several diseases, including neurodegeneration and various malignancies. However, despite intensive research in this area, key questions remain unanswered concerning intracellular protein degradation. Among these are the modes of specific and timed recognition for the degradation of the many substrates, and the mechanisms that underlie aberrations in the system that lead to pathogenesis of diseases.

Mechanisms of Ubiquitination and Degradation

Ubiquitination

Degradation of a protein via the UPS involves two discrete and successive steps (see Figure 1 for a general scheme of the UPS). The protein substrate is first tagged by covalent attachment of multiple ubiquitin molecules to generate the polyubiquitin chain that serves as a recognition marker to the downstream 26S proteasome complex. The tagged protein is then degraded by the **26S proteasome complex** (Prod. No. **P 3863**) leading to the subsequent release of free and reusable ubiquitin [1-3]. This last process is mediated by ubiquitin recycling enzymes such as **isopeptidases** (Prod. No. **I 1154**), also known as ubiquitin-specific proteases (UBPs) and deubiquitinating enzymes (DUBs).

Conjugation of **ubiquitin** (Prod. No. **U 6253**), a highly evolutionarily conserved 76 amino acid residue polypeptide, to the protein substrate proceeds via a three-step cascade mechanism. Initially, the ubiquitin-activating enzyme, E1, activates ubiquitin in an ATP-requiring reaction to generate a high-energy thiol ester intermediate, E1-S~ubiquitin. One of several E2 enzymes known as ubiquitin-carrier proteins or **ubiquitin-conjugating enzymes** (UBCs, Prod. No. **U 8382**) then transfer the activated ubiquitin from E1 via an additional high-energy thiol ester intermediate, E2-S~ubiquitin, to the substrate that is specifically bound to a member of the ubiquitin-protein ligase family, E3, of which a number of different classes are known to exist. For the HECT (*H*omologous to the *E*6-AP *C*-*T*erminus) domain E3s, the ubiquitin is transferred once again from the E2 enzyme to an active site cysteine residue on the E3 to generate a third high-energy thiol ester intermediate, E3-S~ubiquitin, prior to its transfer to the ligase-bound substrate. RING finger-containing E3s catalyze direct transfer of the activated ubiquitin moiety to the E3-bound substrate. E3s catalyze the last step in the conjugation process, namely the covalent attachment of ubiquitin to the substrate. The ubiquitin molecule is generally transferred to an ε-NH_2 group of an internal lysine residue in the substrate to generate a covalent isopeptide bond. In some cases, however, ubiquitin is conjugated to the N-terminal amino group of the substrate. By successively adding activated ubiquitin moieties to internal lysine residues on the previously conjugated ubiquitin molecule (mostly to Lys^{48}), a polyubiquitin chain is synthesized that is subsequently recognized by the downstream 26S proteasome complex. Thus, E3s play a key role in the ubiquitin-mediated proteolytic cascade by serving as the specific recognition factors of the system. In certain cases, the first ubiquitin moiety is conjugated to the substrate by one E3, while chain elongation is catalyzed by a different ligase often termed E4. Certain substrates are conjugated by a single ubiquitin moiety in a three-step mechanism similar to the attachment of ubiquitin that occurs during polyubiquitination. Monoubiquitinated substrates are not degraded as the single ubiquitin moiety is not recognized by the 26S proteasome complex. Rather, the single ubiquitin moiety serves as a routing signal that, in most cases, targets membrane proteins to the lysosome/vacuole. For recent reviews on E3s and ubiquitination, see [4-7] and on the role of monoubiquitination, see [8].

About the Author

Dr. Aaron Ciechanover received his M.D. from "Hadassah" Medical School of the Hebrew University in Jerusalem and a D.Sc. in Biochemistry from the Technion in Haifa, Israel. While a Ph.D. student with Dr. Avram Hershko, the two discovered that covalent attachment of ubiquitin to the target substrate signals it for degradation. They also discovered the three enzymes, E1, E2, and E3, that catalyze conjugation, and elucidated their mode of action. As a postdoctoral fellow in the laboratory of Dr. Harvey Lodish at the Massachusetts Institute of Technology, Cambridge, MA, USA, he also worked with Drs. Alexander Varshavsky and Daniel Finley. The three have elucidated the enzymatic aberration in a cell cycle arrested mutant as a defect in E1, and used this model to demonstrate that the ubiquitin system is involved in targeting short-lived proteins in cells. He is currently a distinguished professor in the Department of Biochemistry and the Center of Tumor and Vascular Biology in the Bruce Rappaport Faculty of Medicine and the Rappaport Institute at the Technion, Haifa Israel. His research focuses on the regulation of transcriptional factors by the ubiquitin system.

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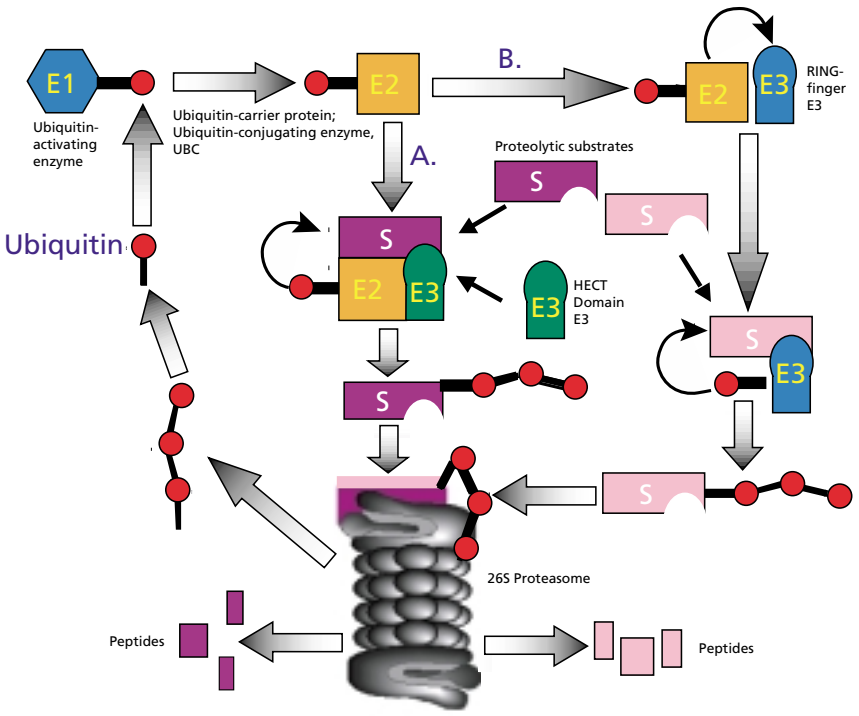

Figure 1. The ubiquitin-proteasome system. Ubiquitin is first activated by the ubiquitin-activating enzyme, E1, to a high energy thiol ester intermediate. It is then transferred, still as a high energy intermediate, to a member of the ubiquitin-carrier proteins family of enzymes, E2 (also known as a ubiquitin-conjugating enzyme - UBC). From E2 it can be transferred directly to the substrate, S, that is bound specifically to a member of the ubiquitin ligase family of proteins, E3. This occurs when the E3 belongs to the RING finger family of ligases (A). In the case of an HECT (Homologous to the E6-AP C-Terminal domain) domain-containing E3 ligase (B), the activated ubiquitin moiety is transferred first to the E3, to generate yet another high-energy thiol ester intermediate, before it is transferred to the E3-bound target substrate. Additional ubiquitin moieties are added successively to the previously conjugated one in a similar mechanism to generate a poly-ubiquitin chain. The poly-ubiquitinated substrate is bound specifically to the 26S proteasome complex. The substrate is degraded to short peptides, and free and reusable ubiquitin is released via the activity of de-ubiquitinating enzymes (DUBS).

Degradation

Degradation of polyubiquitinated substrates is carried out by a large, ~2 mega dalton protease complex, referred to as the 26S proteasome, that does not recognize non-modified substrates. In one established case, that of the polyamine-synthesizing enzyme ornithine decarboxylase (ODC), the proteasome recognizes and degrades the substrate without prior ubiquitination. The proteasome is a multicatalytic protease that degrades polyubiquitinated proteins to short peptides. It is composed of two sub-complexes: a **20S core particle** (CP, Prod. No. P 3988) that carries the catalytic activity, and a regulatory 19S regulatory particle (RP). The 20S CP is a barrel-shaped structure composed of four stacked rings; two identical outer α rings and two identical inner β rings. The eukaryotic α and β rings are each composed of seven distinct subunits, giving the 20S complex the general structure of $\alpha_{1\text{-}7}\beta_{1\text{-}7}\beta_{1\text{-}7}\alpha_{1\text{-}7}$. The catalytic sites are localized to some of the β subunits. Most of the 20S complexes are capped by two 19S complexes, one on each extremity. However, many have one 19S particle on one side and a different complex, **PA28** (Prod. No. P 5988) for example, on the other.

The 19S RP can be further dissected into two multisubunit sub-complexes referred to as the lid and the base. The base, which generates a direct contact with the α ring of the 20S complex, is made up of six homologous ATPases (Rpt1-6) together with three non-ATPase subunits (Rpn1, 2, and 10). The lid of the RP is made of eight non-ATPase subunits (Rpn 3-9), which can be released from the proteasome or can rebind under certain conditions. Altogether, the 19S RP comprises 17 subunits. One important function of the 19S RP is to recognize ubiquitinated proteins and other potential substrates of the proteasome. Two ubiquitin-binding subunits of the 19S RP have been identified, referred to as Rpn10 (S5a in mammalian cells) and Rpt5 (S6'). However, their importance and mode of action have yet to be determined. A second function of the 19S RP is to open an orifice in the α ring that will allow entry of the substrate into the proteolytic chamber. Also, since a folded protein would not be able to enter through the narrow proteasomal channel, it is assumed that the 19S particle unfolds substrates and inserts them into the 20S CP. Both the channel opening function and the unfolding of the substrate require metabolic energy, which is probably provided by ATP and the six base ATPase subunits.

Following degradation of the substrate, short peptides derived from the substrate are released, along with reusable ubiquitin. These peptides are further degraded to amino acids by cytosolic amino- and carboxypeptidases (for recent reviews on the proteasome, see Refs. 9-11). A

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small fraction of these peptides are translocated across the endoplasmic reticulum (ER) membrane, bind to MHC class I molecules and are presented to cytotoxic T cells. In the case of a "foreign" peptide, one that is derived from a viral protein for example, the T cell lyses the presenting cell. Thus, one function of the ubiquitin system is to generate antigens presented on MHC class I molecules (for a recent review on generation of antigens by the UPS, see Ref. 12). It should be noted, however, that proteasomal degradation is not always complete. In some cases, the proteasome, rather than completely destroying its target, processes the ubiquitinated substrate precisely, releasing a truncated product. In the case of the NF-κB transcriptional regulator, an active subunit (**p50**, Prod. No. **N 9909**, or p52) is thus released from a longer inactive precursor (p105 or p100) [13].

Ubiquitin-like proteins, ubiquitin-interacting motifs and ubiquitin-associated domains

Both enzymes and substrates of the ubiquitin system can be modified by *UB*iquitin-*L*ike (UBL) proteins. This modification, similar to mono-ubiquitination, occurs only once. Modification of enzymes affects their activity. Thus, modification of the Cullin component of the SCF (*S*kp2, *C*ullin, *F*-box protein) E3 complexes by the ubiquitin-like protein **NEDD8** (Prod. No. **N 8529**) increases the affinity of the E3 ligases to the E2 component of the conjugation machinery, thus stimulating their conjugating activity. In the case of protein substrates, modification can affect their availability to the ubiquitination/degradation machinery and consequently their cellular stability. For example, in the case of IκBα, the inhibitor of the transcriptional regulator NF-κB, modification by **SUMO-1** (Prod. Nos. **S 0314** and **S 0439**) that targets the same lysine residues targeted by ubiquitin, appears to protect the substrate from cytokine-induced ubiquitination and subsequent degradation. In a completely different case, SUMOylation of RanGAP1 targets the protein to the nuclear pore complex. Modification by ubiquitin-like proteins involves a similar mechanism to that of ubiquitination, yet, the conjugating enzymes are different, and the two sets are not interchangeable (for recent reviews on modification by UBLs, see Refs. 14,15).

A recently discovered group of proteins are those that carry a UBL domain, such as the ubiquitin ligase Parkin, and the proteins that have a *U*biquitin *I*nteracting *M*otif (UIM) or a *UB*iquitin-*A*ssociated (UBA) domain. The UBL domain that is part of other proteins cannot be conjugated, as it lacks a free Arg-Gly-Gly C-terminal domain required for conjugation of ubiquitin and UBLs that have the capacity to modify proteins. The UBL domain serves, most probably, to facilitate interaction with other ubiquitin-binding proteins of the system, such as the proteasome. This interaction may enhance the efficiency of the proteolytic process by bringing together different enzymatic/ancillary elements of the system. Therefore, the UIM- and UBA-containing proteins serve as recognition elements in *trans*, binding ubiquitinated proteins and linking them to other elements of the system (for a recent review on UIMs and UBAs, see Refs. 16,17).

Substrate Recognition

A major unresolved question concerns how the UPS achieves its high specificity and selectivity. Why are certain proteins extremely stable in the cell, while others are extremely unstable and short-lived? Why are certain proteins degraded only at a particular time point during the cell cycle or only following specific extracellular stimuli, yet are stable under most other conditions? It appears that the specificity of the UPS is determined by two distinct and unrelated groups of proteins, specifically E3s and ancillary proteins. First, within the ubiquitin system, substrates must be recognized and bind to a specific E3 before they are ubiquitinated. In a few cases, the target substrates will be recognized in a constitutive manner. However, in most cases they must undergo a post-translational modification, via phosphorylation or oxidation, for example, or a change in their 3-dimensional structure in order to bind to the ligase. In some cases, the substrates will not be recognized directly and will only bind to the E3 via a third protein, a recognition element in *trans*. Thus, the human Papillomavirus, HPV, codes an oncoprotein, E6, that binds to p53 and targets it for degradation by the cellular ligase E6-AP (*E6-A*ssociated *P*rotein) (see below). The stability of other proteins depends on association with molecular chaperones that also act as recognition elements in *trans* and serve as a link to the appropriate ligase. In some other instances, the E3 must be switched on by undergoing post-translational modification in order to recognize and bind substrate. Thus, the *A*naphase *P*romoting *C*omplex (APC), or the c-Cbl ligase must be phosphorylated in order to recognize their substrates, certain cell cycle regulators and the EGF receptor, respectively. Other proteins, such as certain transcription factors, have to dissociate from the specific DNA sequence to which they bind in order to be recognized by the system. Stability of yet other proteins depends on oligomerization.

Thus, in addition to the E3s themselves, modifying enzymes (such as kinases), ancillary proteins, or DNA sequences to which substrates bind, also play an important role in the recognition process (the different recognition modes are described in Refs. 1 and 3-7). An interesting case involves cell and ER membrane proteins. Membrane proteins, mostly receptors, but also ion channels, are ubiquitinated either constitutively or in response to ligand engagement, and are transported via a series of vesicles for degradation in the lysosome/vacuole. Ubiquitination occurs on the cytosolic tail of these proteins. Involvement of the ubiquitin system occurs in multiple steps along the pathway, from cell surface ubiquitination of the protein substrates through generation of multiple vesicular bodies (MVBs; reviewed in Ref. 18). Misfolded, unassembled, or an otherwise abnormal ER membrane and even lumenal proteins are transported in retrograde via the translocation channels in the ER membrane and are polyubiquitinated and degraded by the proteasome on the cytosolic surface of the ER. In yeast, but also in mammals, the ubiquitination enzymes E2 and E3 appear to be associated directly (membrane proteins) or indirectly (via recruiting proteins that are themselves membrane proteins) with the ER membrane. The machinery

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involved in this process, including the lumenal chaperones that assist in unfolding the proteins and translocating them across the membrane, in retrograde, has been designated ERAD- *ER-A*ssociated *D*egradation (see Ref. 19).

Functions and Substrates of the Ubiquitin System

Ubiquitin-mediated proteolysis of a variety of cellular proteins plays an important role in many basic cellular processes. Among those are, 1) regulation of cell cycle and division, differentiation and development, 2) involvement in the cellular response to stress and extracellular effectors, 3) morphogenesis of neuronal networks, 4) modulation of cell surface receptors, ion channels and the secretory pathway, 5) DNA repair, 6) transcriptional regulation, 7) transcriptional silencing, 8) long-term memory, 9) circadian rhythms, 10) regulation of the immune and inflammatory responses, and 11) biogenesis of organelles. Not surprisingly, the list of cellular proteins that are targeted by ubiquitin is growing rapidly. Among them are cell cycle regulators such as cyclins, cyclin-dependent kinase inhibitors, and proteins involved in sister chromatid separation, tumor suppressors, transcriptional activators and their inhibitors. Cell surface receptors and ER proteins are also targeted by the system. Finally, mutated and denatured/misfolded proteins are recognized specifically and are removed efficiently. Accordingly, the UPS is a key player in cellular quality control and defense mechanisms.

Regulation of the Ubiquitin System

The UPS can be regulated at the level of ubiquitination or at the level of proteasome activity. Since conjugation and proteasomal degradation is required for a multitude of cellular functions, regulation must be delicately and specifically tuned. In a few cases, general rather than specific components of the pathway can be modulated by physiological signals. For example, upregulation of the pathway is observed during the massive degradation of skeletal muscle proteins that occurs during normal fasting, but also under pathological conditions such as cancer-induced cachexia, severe sepsis, metabolic acidosis, or following denervation. In most cases, however, regulation is specific and the target-regulated substrates are recognized, following a specific modification, by specific ligases that bind to defined motifs. The targeting motif can be a single amino acid residue (e.g., the N-terminal residue) or a sequence (the Destruction box in cyclins) or a domain (such as a hydrophobic patch) that is not normally exposed. In other cases, the motif is generated by a post-translational modification such as phosphorylation that may occur in response to cell needs or external signals. Phosphorylation can occur either on the substrate or the ligase.

Ubiquitination and Pathogenesis of Human Diseases

While inactivation of a major enzyme such as E1 is obviously lethal, mutations or acquired changes in enzymes or in recognition motifs in substrates that do not affect vital pathways or that affect the involved process only partially, may result in a broad array of phenotypes.

Proteasome/Ubiquitin Related Research Tools Available from Sigma-RBI

Ubiquitin and Proteases

C 0483 **Z-Leu-Leu-Glu-7-amido-4-methylcoumarin**
C 0608 **Z-Leu-Leu-Leu-7-amido-4-methylcoumarin**
I 1154 **Isopeptidase, Ubiquitin C-Terminal Hydrolase, Ubiquitin Thiolesterase**
N 8529 **Nedd8, human, recombinant**
N 2030 **Nedd8 Precursor, human, recombinant**
P 5988 **PA28 Activator**
P 3988 **20S Proteasome, rabbit**
P 3863 **26S Proteasome Fraction, rabbit**
F 4051 **Proteasome Fraction II, rabbit reticulocyte cell extract**
F 3926 **Proteasome Fraction II Hela S3 cell extract human**
P 4113 **Proteasome Fraction from Hela cell extract**
S 0314 **SUMO-1/Sentrin-1, human, recombinant**
S 0439 **SUMO-1/Sentrin-1, human, recombinant, GST-tagged**
U 5632 **Ubiquitin-Agarose**
U 1507 **Ubiquitin Aldehyde**
U 6253 **Ubiquitin, bovine**
U 9382 **Ubiquitin-carrier protein H10, human, recombinant UbcH10**
U 8757 **Ubiquitin-carrier protein H5b, human, recombinant UbcH5b**
U 8882 **Ubiquitin-carrier protein H5c, human, recombinant UbcH5c**
U 9007 **Ubiquitin-carrier protein H6, human, recombinant UbcH6**
U 9132 **Ubiquitin-carrier protein H7, human, recombinant UbcH7**
U 9257 **Ubiquitin-carrier protein H9, human, recombinant UbcH9**
U 8382 **Ubiquitin Conjugating Enzyme Fractions, rabbit**
U 5504 **Ubiquitin fluorescein conjugate**
U 5382 **Ubiquitin, human, recombinant, N-terminal FLAG-tagged**
U 5507 **Ubiquitin, human, recombinant, N-terminal his-tagged**
U 8507 **Ubiquitin K48R, human**
U 1632 **Ubiquitin, methylated, bovine**

Antibodies

N 2786 **Anti-NEDD**
E 3152 **Anti-E1 Ubiquitin Activating Enzyme**
U 5379 **Rabbit anti-Ubiquitin**

Calpain and Proteasome Inhibitors

C 0788 **CBZ-Leu-Leu-Glu-β-naphthylamide**
C 9857 **CBZ-Leu-Leu-Leu-B(OH)$_2$,** Proteasome Inhibitor III
C 2211 **N-CBZ-Leu-Leu-Leucinal**
C 6706 **N-CBZ-Leu-Leu-Norvalinal**
L 6785 **Lactacystin**
L 7035 **clasto-Lactacystin β-lactone**
C 0358 **Z-Leu-Leu-Phe-CHO**
C 8984 **Z-Leu-Leu-Leu-fluoromethyl ketone**

One can conceive of two main groups of pathological states that may be associated with aberrations in the ubiquitin system; first, those that result from loss of function, for example, a mutation in a ubiquitin system

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enzyme or target substrate that results in stabilization of certain proteins with an increase in their level and, secondly, those that result from gain of function, for example destabilization of the target substrates with a decrease in their level that results from abnormally accelerated degradation.

Malignancies

Alterations in ubiquitination and deubiquitination reactions have been directly implicated in the etiology of many malignancies. In general, cancers can result from stabilization of oncoproteins or destabilization of tumor suppressor gene products. Some of the natural substrates for degradation by the proteasome are oncoproteins that, if not properly removed from the cell, can promote cancer. For instance, ubiquitin targets N-myc, c-myc, c-fos, c-jun, Src, and the adenovirus E1A proteins. Destabilization of tumor suppressor proteins such as p53 and p27 has been implicated in the pathogenesis of malignancies (on the linkage between aberrations in the ubiquitin system and malignancies, see Ref. 20).

In one fascinating case, that of uterine cervical carcinoma, the level of the tumor suppressor protein p53 is extremely low. Most of these malignancies are caused by high-risk strains of the human papillomavirus (HPV). Detailed studies have shown that the suppressor is targeted for ubiquitin-mediated degradation by the virally encoded oncoprotein E6. Degradation is mediated by the native HECT domain E3 enzyme E6-AP, where E6 serves as an ancillary protein that allows recognition of p53 in *trans*. E6-AP will not recognize p53 in the absence of E6. E6 associates with both the ubiquitin-ligase and the target substrate and brings them to the necessary proximity that is assumed to allow catalysis of conjugation to occur. Removal of the suppressor by the oncoprotein is probably an important mechanism used by the virus to transform cells.

Similar to the case of p53, low levels of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ have been demonstrated in colorectal, prostate and breast cancers. p27 acts as a negative growth regulator/tumor suppressor. Its level is high in quiescent cells, but following mitogenic stimuli, it is rapidly degraded by the ubiquitin system, allowing the CDK/cyclin complexes to which it was bound to drive the cell into the S phase. As noted, the level of p27 is markedly reduced in several cancers, including breast, colorectal, and prostate carcinomas, and in many of these cases a strong correlation exists between the low level of p27 and the aggressiveness of the disease. Levels of p27 have become an important and novel prognostic factor for survival, recurrence, and evaluation of therapy, where extremely low expression predicts poor prognosis. Dissection of the mechanism that underlies the decrease in p27 revealed that the rapidly degraded p27 is of the wild type (WT) species and it is probably abnormal activation of the ubiquitin system that leads to accelerated degradation of the suppressor. Mechanistic analysis revealed that the low level of p27 correlates directly with increased level of Skp2, the F-box protein involved in p27 ubiquitination, and that ectopic overexpression of Skp2 in experimental animals is oncogenic.

Mutations in components of the ubiquitination machinery can also cause malignancies. Mutations in the von-Hippel Lindau (*VHL*) gene predisposes individuals to a wide range of malignancies, including the vast majority of sporadic cases of renal cell carcinoma, pheochromocytoma, cerebellar hemangioblastomas, and retinal angiomas. A hallmark of $VHL^{-/-}$ tumors is the high degree of vascularization that arises from constitutive expression of hypoxia inducible genes including the switch transcription factor hypoxia-inducible factor-1α (HIF-1α) and the crucial vascular endothelial growth factor (VEGF). The protein coded by VHL, pVHL, is a subunit in a ubiquitin ligase complex that is involved in targeting of HIF-1α for ubiquitin- and proteasome-mediated degradation. Under normoxic conditions, HIF-1α is hydroxylated specifically on Pro^{564} to generate a hydroxyproline derivative, in a reaction that involves molecular oxygen and soluble prolyl-4-hydroxylase. This hydroxylated Pro residue is recognized by the pVHL E3 complex that targets the molecule for ubiquitination and subsequent degradation. Under hypoxic conditions, HIF-1α is stable, as the efficiency of the hydroxylation reaction under these conditions is extremely low. Loss of VHL function stabilizes HIF-1α which may explain the stimulation of vascular growth in tumors in which VHL is mutated or lacking. Since overexpression of VEGF alone or many of the other known target proteins of HIF does not lead to malignant transformation, and since WT VHL can restore normal growth control in these malignant cells, researchers assume that pVHL and/or HIF must have additional, yet unknown substrates/target genes.

Neurodegenerative Disorders

Accumulation of ubiquitin conjugates and/or inclusion bodies associated with ubiquitin, proteasome and certain disease-characteristic proteins have been reported in a broad array of chronic neurodegenerative diseases. These include the neurofibrillary tangles of Alzheimer's disease (AD), brainstem Lewy bodies (LBs), the neuropathological hallmark in Parkinson's disease (PD), and nuclear inclusions in CAG repeat expansion (poly-glutamine extension) disorders such as Huntington's disease. However, in all these cases, a direct pathogenetic linkage to aberrations in the ubiquitin system has not been established. One factor that complicates the establishment of such linkage is the realization that many of these diseases, such as Alzheimer's and Parkinson's, are not defined clinical entities, but rather syndromes with different etiologies. The pathogenetic significance of these aggregates has therefore remained enigmatic. Recent findings demonstrate that soluble aggregated proteins can inhibit the ubiquitin system [21]. However, an emerging concept proposes that the sequestration of the aggregated proteins from the cytosol and nucleoplasm, and their concentration in defined inclusion bodies, separates them from sensitive cellular machineries, such as the transcriptional apparatuses, and is therefore protective. Thus, it is the soluble fraction of the aggregated proteins that is toxic.

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The case of Parkinson's disease highlights the complexity of the involvement of the ubiquitin system in the pathogenesis of neurodegeneration. While the pathogenetic base of the sporadic, late onset PD, the most prevalent form of the disease, has remained elusive, certain lessons may be learned from the identification of the defect in several rare, early onset, forms of familial PD. One important player in the pathogenesis of PD is Parkin which is a RING-finger E3. Mutations in the gene appear to be responsible for the pathogenesis of autosomal recessive juvenile parkinsonism (AR-JP), one of the most common familial forms of PD. Parkin ubiquitinates and promotes the degradation of several substrates. Among them are CDCrel-1 which appears to play a role in transmitter release; synphilin-1 that along with α-synuclein may be involved in synaptic vesicle formation and function; and the Pael receptor, a putative G protein-coupled transmembrane polypeptide, the accumulation of which leads to ER stress. It is possible that aberrations in the degradation of one or more of these substrates that lead to their accumulation is neurotoxic and underlies the pathogenesis of AR-JP.

In the late 1990s, it was reported that two mutations in the N-terminal domain of α-synuclein, a protein that is most probably involved in dopamine neurotransmission/release via effects on vesicular storage, are associated with a rare form of autosomal dominant familial PD. The mutant proteins have a higher tendency to generate fibrils compared with their WT counterpart. The autosomal dominant nature of the disease associated with the mutant forms of α-synuclein strongly suggests that a gain-rather then a loss-of-function mutation underlies the mechanism of disease formation. It should be noted that overexpression of the WT form of α-synuclein in neurons induced apoptosis or sensitized the cells to toxic agents. The linkage between α-synuclein and the ubiquitin system is not that clear. It has been shown that the protein is targeted by the proteasome, and that the mutant form is slightly less susceptible to degradation. An important finding is that aggregated and even monomeric α-synuclein binds to the S6' proteasomal protein and inhibits proteasomal function. This aggregation, which is the primary event, may lead to secondary damage by inhibiting the UPS (for the linkage of aberrations in the ubiquitin system to neurodegeneration, see Refs. 22 and 23).

Cystic Fibrosis

The cystic fibrosis gene encodes the CF transmembrane regulator (CFTR) that is a chloride channel. Only a small fraction of the protein matures to the cell surface. Most of the protein is degraded prior to its maturation from the ER by the ubiquitin system. One frequent mutation in the channel is ΔF508. The mutation leads to an autosomal recessive inherited multisystem disorder characterized by chronic obstruction of airways and severe maldigestion due to exocrine pancreatic dysfunction. Despite normal ion channel function, $CFTR^{\Delta F508}$ does not reach the cell surface, but rather, it is retained in the ER and degraded, via ERAD, by the ubiquitin system. It is possible that the rapid and efficient degradation results in the complete lack of cell surface expression of the F508 protein, and therefore contributes to the pathogenesis of the disease.

Drug Development

Because of the central role the ubiquitin system plays in such a broad array of basic cellular processes, development of drugs that modulate the activity of the system may be difficult. Inhibition of enzymes common to the entire pathway, such as the proteasome, may affect many processes nonspecifically, although, a narrow window between beneficial effects and toxicity might be identified for a short-term treatment. Recent experimental evidence strongly suggests that such inhibitors may indeed be beneficial in certain pathologies, such as in multiple myeloma, a malignancy that affects the bone marrow (see for example refs. 24-25). While the mechanism of action of the general inhibitor is not clear, it was suggested that inhibition of the ubiquitin system leads, via different mechanisms, to re-sensitization of the malignant cells to apoptosis via inhibition of activation of NF-κB.

A completely different approach to drug development involves the design of small molecules that interfere with the activity of specific E3s. For example, peptides that bind specifically to HPV-E6 prevent its association with p53 and interfere with its targeting by E6-AP. Such peptides were able to induce p53 in HPV-transformed cells with subsequent reversal of certain malignant characteristics and induction of apoptosis. Similarly, one can think of small molecules that mimic the phosphorylated and recognition domain of p27 and will interfere with its accelerated degradation by Skp2.

Concluding Remarks

Remarkable progress has been made in our understanding of how proteins are targeted for degradation and how ubiquitin-mediated proteolysis is involved in regulating numerous cellular processes. However, much of the ubiquitin system remains undiscovered. In particular, the complete cohort of substrates of the system, the identity of their ligases, and their mode of specific recognition still remain elusive. In addition, for many pathological states, we still do not know whether the system plays a primary role, or whether the primary pathology impairs its function in a secondary manner, thus exacerbating the pathology. This lack of knowledge impedes the development of agents to target the degradation of specific proteins. It is only the gradual unraveling of the complete scope of the system that will lead to its utilization as a platform for the development of mechanism-based agents and drugs for use in both biological and clinical applications.

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New Product Highlights

L-765,314: A potent and selective α_{1B} adrenoceptor antagonist

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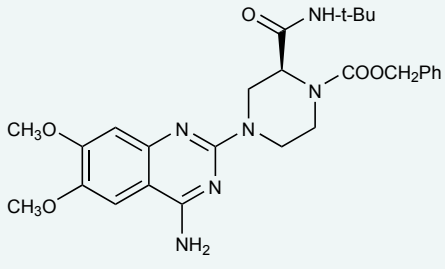
α_1-Adrenoceptors are a family of G protein-coupled receptors that mediate responses to **epinephrine** (Prod. Nos. **E 4250** and **E 4375**) and **norepinephrine** (Prod. No. **A 9512**). Three subtypes exist, referred to as α_{1A}, α_{1B} and α_{1D}, and are known to play a role in many physiological processes including smooth muscle contraction, myocardial inotropy and chronotropy, and hepatic glucose metabolism [1]. α_1-Adrenoceptors are expressed in both cardiovascular organs and in the central nervous system, where the roles of the specific subtypes remain to be determined. The α_{1B} subtype is composed of 519 amino acid residues and is equally responsive to epinephrine and norepinephrine, which activate downstream targets through coupling to $G\alpha_{q/11}$ [1].

Sigma-RBI is pleased to offer the potent and selective α_{1B} adrenoceptor antagonist **L-765,314** (Prod. No. **L 3040**). In receptor binding assays using cloned human α_{1A}, α_{1B} and α_{1D} adrenoceptors, L-765,314 displayed a K_i value of 2.0 nM at the α_{1B} adrenoceptor, showing 210-fold and 17-fold selectivity over α_{1A} and α_{1D} adrenoceptors, respectively [2]. In tissue binding assays, L-765,314 displayed nanomolar potency and selectivity that corresponded to the predominant α_1 subtype expressed in the tissue [2]. Recently, L-765,314 has been used to study periarterial nerve electrical stimulation (PNS) in canine splenic artery, leading to the conclusion that PNS-induced responses are mediated via the α_{1B} adrenoceptor subtype [3].

Accordingly, L-765,314 will prove to be a useful tool for delineating the specific functions of the α_{1B}-adrenoceptor subtype in various tissues and will be helpful in defining the therapeutic potential of subtype-selective adrenergic drugs.



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