Ubiquitin – the kiss of death goes Nobel. Will you be quitting?

Behuliak M, Celec P, Gardlik R, Palffy R

BiomeD Research & Publishing Group, Bratislava, Slovakia. **michalbehuliak@gmail.sk**

Abstract

The Nobel prize in chemistry 2004 was given to Aaron Ciechanover, Avram Hershko and Irwin Rose for their discovery of the ubiquitin mediated proteolysis. Years of research have shown that the ubiquitin pathway plays a crucial role in the cellular metabolism and its regulation. These scientists together with Alexander Varshavsky have identified the most important elements of this pathway as well as their interactions. The ubiquitin pathway degrades intracellular proteins with an ubiquitin chain being the tag that marks proteins assigned for degradation. This process is mediated by ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin-protein ligases (E3). Mono-ubiquitination and deubiquitination play a classic regulatory role in numerous processes including cell-cycle, transcription, translation, DNA repair, stress response etc. This article tries to summarize current knowledge on the molecular basis of the ubiqutin pathway. ***(Fig. 1, Ref. 52.)***
Key words: ubiquitin, proteasome, proteolysis, ubiquitination, deubiquitination.

The Nobel prize in chemistry 2004 was given to Aaron Ciechanover, Avram Hershko and Irwin Rose for their discovery of the ubiquitin mediated proteolysis and its significance. What makes this pathway so important? Firstly, it is its nearly ubiquitous occurrence in all eukaryotic cells and the related evolutionary conserved characteristics. However, the lately discovered role of ubiquitin and ubiquitination in signaling pathways and regulation has made this pathway much more than only a waste disposal for the cell. Although the basic principles of functioning are more or less uncovered, the ubiquitination as a posttranslational modification of proteins is a hot topic for scientists in molecular biomedicine.

Proteolysis

The knowledge on proteolytic processes outside of the cells is clinically used for years (e.g. pancreatic proteases, matrix metalloproteases etc.), however, the information about intracellular proteolysis with its huge potential is only at the beginning of clinical use. The dynamic turnover of proteins in the cells, a precise balance of synthesis and degradation, was discovered by Rudolf Schoenheimer in 1942, when he first used radio-labeling of proteins in vivo. Previously, the proteins in organisms were viewed as stable components (an exception were only dietary proteins) and even years after this discovery intracellular proteolytic pathways were thought to serve largely to removal of aged or damaged proteins (Ciechanover, 2005). Repressors and activators of gene expression were considered to be the main long-lived factors of cellular regulation. Many regulators in the cell are short-lived proteins and their levels are maintained by a dynamic turnover (Finley et al, 2004). The eukaryotic cell has three different proteolytic pathways for the degradation of proteins. The lysosome was the first eukaryotic proteolytic system discovered. It was hypothesized that the degradation of intracellular proteins is being realized in the lysosome. Two decades were necessary to accumulate data, which has led to the conclusion that the intracellular proteolysis must be non-lysosomal. One of the crucial steps was the identification of specific inhibitors of lysosomal proteases. These inhibitors have different effects on degradation of intracellular and endocytosed extracellular

BiomeD research & publishing group, Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: M. Behuliak, Nabrezna 1001/15, SK-017 01 Povazska Bystrica, Slovakia

Acknowledgment: The authors would like to thank J. Christophoryova for cooperation. The work was supported by Grant of the Comenius University 116/2004 and 117/2004 and the Grant Agency for Science and Technology APVT-20-003104.

proteins. The inhibition of degradation was significant (obvious) in the case of extracellular proteins but not significant for intracellular short-lived and abnormal proteins. The degradation of long-lived proteins was partially inhibited (Ciechanover, 2005). The second main argument against the intracellular proteolysis mediated by lysosome was the termodynamically paradoxical observation that the proteolysis of intracellular proteins is ATP-dependent and the lysosomal proteases degrade proteins exergonically. On the basis of these observation the functions of the endosome-lysosome system were revealed and described. It degrades extracellular proteins and cell-surface proteins that are taken up via pinocytosis or receptor-mediated endocytosis (Glickman and Ciechanover, 2002). The second eukaryotic proteolytic system – the ubiquitin-proteasome system was identified later. It degrades proteins from the cytoplasm, nucleus and endoplasmic reticulum. The third proteolytic system in the eukaryotic cell is of bacterial origin. It is responsible for the proteolysis in the semiautonomous organelles – mitochondria and chloroplasts.

Ubiquitin – the molecular view

Ubiquitin (8.5 kDa) is a small heat-stable polypeptide of 76 amino acid residues. It is one of the evolutionarily most conserved proteins. There are only 3 amino acid substitutions between mammalian and yeast amino acid sequences of ubiquitin (Wojcik, 2002). The molecule of ubiquitin is folded up into a globular structure, which is extremely compact and tightly hydrogen-bounded. The major part of the polypeptide chain (87 %) is involved in formation of main secondary structural features which are hydrogen-bounded structures, including 3.5 turns of α-helix, a mixed ß-sheet which contains five strands and seven reverse turns. These main secondary structures (α-helix and ß-sheet) form the hydrophobic core of the protein. Besides, the molecule of ubiquitin contains a several unusual secondary structural features such as a parallel G1 ß-bulge, two reverse Asx turns and a symmetric hydrogen-bonding region, which involves the two helices and two of the reverse turns (Vijay-Kumar et al, 1987).

There are several important amino acid residues in the ubiquitin molecule. They are crucial for the specific functions of ubiquitin. The C-terminal residue glycine (Gly76) is critical for the linkage of ubiquitin to the target proteolytic substrates, for the formation of the polyubiquitin chain and the coupling to the specific nonproteolytic substrates. All of these linkages are identical – an isopeptide bond between Gly76 and an ε-amino group of lysine (Lys) residue (Hershko and Ciechanover, 1998). The difference embodies only the location of this Lys residue. In the ubiquitin-mediated degradation of substrates the initial ubiquitin is linked to Lys of substrates and creates a nidus of polyubiquitination. The polyubiquitin chain is synthesized on this initial ubiquitin and provides a signal for proteolysis by the proteasome. In this polyubiquitin chain, Gly76 of one ubiquitin moiety is linked to the internal Lys48 of the previously linked ubiquitin moiety (Hershko and Heller, 1985; Chau et al, 1989). An identical signal for degradation of substrate provides the linkage between Gly76 and Lys29. Unlike polyubiquitination via Lys48 and/or Lys29, monoubiquitination and polyubiquitination via Lys63 do not represent the signal for degradation but regulate some of the cell processes (Pickart, 2000).

The ubiquitin system was studied in *S. cerevisiae* at first. In 1984, the first ubiquitin gene, UBI4, was cloned by Finley, Özkaynak and Varshavsky (Ozkaynak et al, 1984). They have found that it encodes a polyubiquitin precursor protein. Later, they have showed this gene was strongly induced by different kinds of stress and that a deletion of this gene resulted in a phenotype hypersensitive to every noxious treatment like heat or oxidative stress (Finley et al, 1987). Later, 3 more ubiquitin genes UBI1, UBI2 and UBI3 were identified. These genes also encode fusion proteins, that are processed to ubiquitin as in UBI4. But these fusion proteins are processed to ubiquitin and one protein of the large ribosomal subunit, L40A (genes UBI1 and UBI2), and one protein of the small ribosomal subunit, S31 (gene UBI3) (Finley et al, 1989). These ribosomal proteins are called carboxyl extension proteins (CEPs) (Kirschner and Stratakis, 2000). Similar ubiquitin genes, as those in yeasts, were found also in the human genome. Ubiquitin is synthesized either as a polyubiquitin precursor protein (UbB or UbC genes) or as a fusion protein of a single ubiquitin to CEPs (UbA gene). UbA80 (HUBCEP80) and UbA52 (HUBCEP52) are human ubiquitin-CEP genes and the analysis of the yeast and human CEPs has shown that they represent identical components of the ribosome (Jentsch et al, 1991). UbA80 encodes, for ubiquitin fused to ribosomal protein S27a (RPS27a), protein of the small ribosomal subunit, and it is similar to UBI3 yeast gene (Redman and Rechsteiner, 1989). The human gene UbA52 (similar to yeast genes UBI1 and UBI2) encodes fusion proteins of ubiquitin and one protein of large ribosomal subunit, L40 (RPL40) (Webb et al, 1994). Human genes UbB and UbC encode polyubiquitin precursor protein, analogous to the yeast gene UBI4 (Kenmochi et al, 1998). These precursor proteins are cleaved by deubiquitinating enzymes to single ubiquitin moieties and ribosomal proteins.

This small heat-stable protein was named ubiquitin because it was found to be ubiquitously expressed in various cells and tissues in eukaryotes, but its function was unknown (Goldstein et al, 1975). In 1980, A. Hershko and his graduate students A. Ciechanover and Y. Hod, in collaboration with I. Rose, showed that the small heat-stable protein was bound to proteins in reaction that is ATP-dependent. They suggested that this small protein, called ATP-dependent proteolytic factor 1 (APF-1), serves as a tag on the proteins for selective degradation (Ciechanover et al, 1980; Hershko et al, 1980). Later, Wilkinson and co-workers observed that APF-1 and ubiquitin were the same protein (Wilkinson et al, 1980). Ubiquitin was also apparently found in prokaryotes, but recent studies have shown that ubiquitin is not expressed in bacterial cells and the previous identification of expression was probably due to contamination with yeast ubiquitin. Besides, the term ubiquitin is a misnomer because its ubiquity is not as general as was previously thought. Thus, the term ubiquitin has mostly historical reasons (Ciechanover, 2005).

The role of ubiquitin-mediated proteolysis

The substrates of ubiquitin-proteasome pathway are the most short-lived proteins existing in a dynamic state. Many of them are regulatory proteins. The turnover of these proteins plays a pivotal role in the regulation processes in the cell (Schwartz and Ciechanover, 1999). These processes include regulation of the cell cycle, cell division, transcription, endocytosis, modulation of cell surface receptors and ion channels, antigen presentation and degradation of resident or abnormal proteins in the endoplasmic reticulum (Hershko and Ciechanover, 1998). The ubiquitin system plays a crucial role in the differentiation and development of the cell, programmed cell death (Lee and Peter, 2003), morphogenesis of neuronal networks, response to stress (Wilkinson, 1995), DNA repair (Pickart, 2002), immune and inflammatory responses and biogenesis of organelles (Pickart, 2000). A large group of protein substrates, acting as ubiquitin targets, consists of cell cycle regulators such as cyclins, activators and inhibitors of cyclin-dependent kinases and anaphase inhibitors (regulation of cell-cycle); tumor suppressors and growth modulators (regulation of cell division, differentiation and development of the cell); transcriptional activators and inhibitors (gene expression regulation), cell surface receptors, mutant and damaged proteins (degradation). The biogenesis of ribosomes is one of the specific processes, where the effects of ubiquitin system are not mediated by degradation of specific substrates (Hershko et al, 2000). Within mentioned wide spectrum of protein substrates, it is not surprising that malfunctions in several ubiquitin-mediated processes may be implicated in the pathogenesis of several diseases.

The ubiquitin pathway

The ubiquitin-mediated protein degradation pathway involves two successive steps. The initial step, conjugation of ubiquitin to the substrate (ubiquitination) – multiple ubiquitin molecules are covalently attached to the target protein and build a polyubiquitin chain linked via the bonds between Gly76 and Lys48 (or Lys29). The following step of ubiquitin-proteasome pathway (Fig. 1) is a degradation of a tagged protein by the 26S proteasome (Hershko and Ciechanover, 1992; Ciechanover, 1994). The covalent attachment of ubiquitin to the substrate proceeds via a three-step mechanism and requires the action of three enzymes (Hershko et al, 1983). The first step is the activation of C-terminal Gly76 of ubiquitin by the ubiquitin-activating enzyme, E1. In this ATP-dependent step an intermediate molecule of ubiquitin adenylate is created, while PPi is released. Ubiquitin is attached to a cysteine residue (Cys) in an active site of E1 and AMP is released. The second step is a transfer of activated ubiquitin from E1 to Cys residue within an active site of a ubiquitin-conjugating enzyme (ubiquitin-carrier protein, UBC), E2. In the third step, ubiquitin from E2 is covalently attached to Lys of a substrate by a ubiquitin-protein ligase, E3. Basically, there is only one type of E1, but there is a large number of different E2 molecule types (for example, in human approximately 100 E2 enzymes are known) and the number of E3 enzymes is even higher by order of magnitude (Pickart and Rose, 1985). The large number of various E2 and E3 provides the high specificity and selectivity of this system to many types of substrates, because each E3 with its cognate E2 recognizes only substrates with the specific ubiquitination signal.

The ubiquitin molecule is transferred from E1 enzyme to a Cys residue in the active site of E2 enzymes and these may participate in the recognition of the substrate too. Some E2 enzymes play also an important role in specific cellular processes. For example, the proteolysis of "N-end rule" substrates is mediated by the yeast Ubc2/Rad6 and it is also involved in DNA repair (Jentsch et al, 1987). Ubc3/Cdc34 is required for the G1 to S-phase transition, and Ubc4 and Ubc5 are implicated in the proteolysis of many short-lived abnormal and normal proteins (Ciechanover, 1998).

E3 enzymes are necessary for the recognition of specific signals in substrates and ubiquitin is transferred from E2 enzyme to the substrate by two different mechanisms performed by different classes of E3 enzymes. The first class of E3 enzymes represents the HECT (homologous to E6-AP C-terminus) domain E3s. These enzymes use the mechanism, where ubiquitin is transferred from E2 to a Cys in an active site of E3 and then it is bound to a substrate linked by E3. In the second mechanism, the linkage between E3 and ubiquitin is not created. E3 enzyme of this class binds appropriate E2 enzyme, as well as the competent substrate. Subsequently, ubiquitin is transferred directly from the E2 enzyme to the substrate. This mechanism is catalyzed by RING (really interesting new gene) finger domain E3s (Glickman and Ciechanover, 2002). E3 enzymes also catalyze the transfer of ubiquitin from E2 or E3 to a polyubiquitin chain already attached to the substrate. Some E3 enzymes bind the substrates directly, but many protein substrates must undergo posttranslational modifications or are associated with adaptor proteins to be recognized by E3s.

E3 enzymes are divided into several families on the basis of their attributes. The first and the main family is the so-called „N-end rule" E3 family (Bachmair et al, 1986). Here belongs the first E3 enzyme discovered, E3α, and the yeast counterpart Ubr1p. These E3 enzymes contain two specific binding sites that recognize the N-end rule – N-terminal amino acid residues of protein substrates. The first binding site (type I) is specific for basic N-terminal amino acid residues and the second site (type II) is specific for bulky hydrophobic N-terminal residue, and so N-end rule E3s bind directly to the substrates. Furthermore, E3 enzymes of this family also bind some proteins, which do not have N-end rule N-terminal residues (Varshavsky, 1996).

The first member of the second family of E3 enzymes – HECT domain family is E6-AP (E6-associated protein). E6-AP does not bind directly to its substrate, p53, but it binds to p53 via an ancillary protein, E6. Therefore, the degradation of p53 by E6-AP is dramatically increased by human papillomavirus (HPV) E6 oncoprotein, which recognizes both E6-AP and the tumor suppressor p53 (Scheffner et al, 1993). A large family of proteins that contain a HECT-domain was found in many eukaryotes. All of these HECT-proteins have conserved C-terminal domain, which contains a conserved active site Cys. On contrary, the N-terminal region of HECT-proteins is variable and may be involved in the recognition of substrates. Variability of

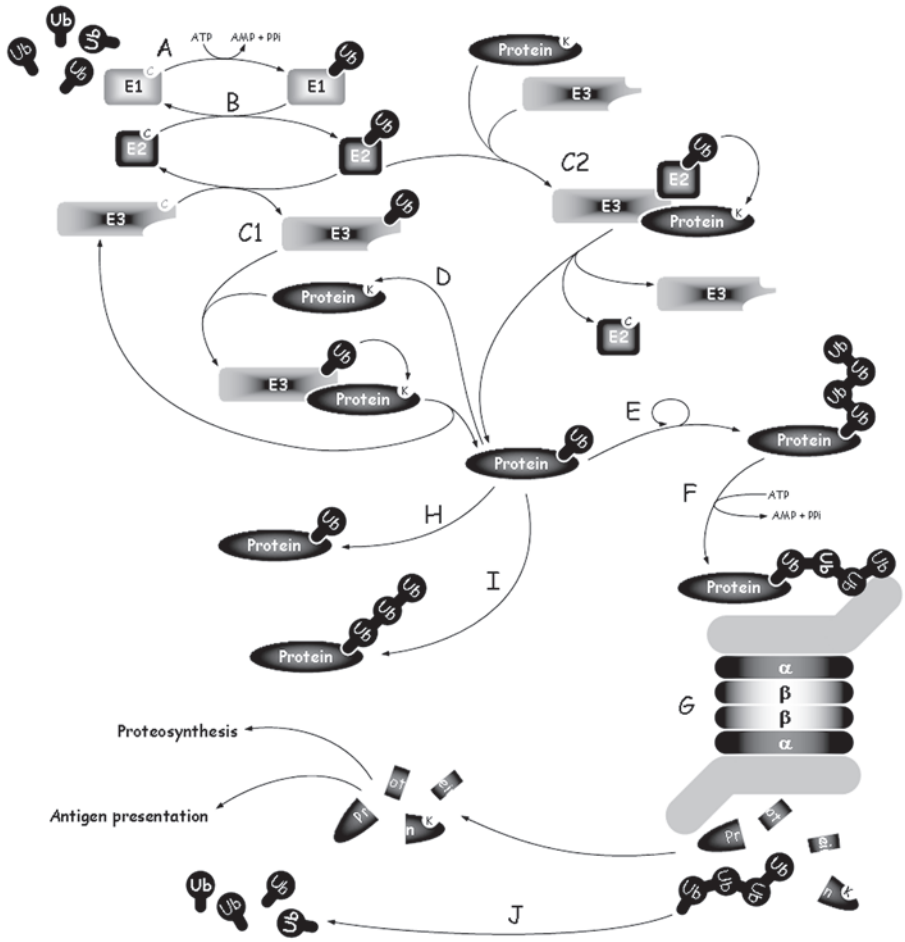

Fig. 1. The ubiquitin-proteasome pathway. Ubiquitin is activated in an ATP-dependent step by E1 (A). Activated ubiquitin is transferred from E1 to E2 (B). Ubiquitin is transferred from E2 to E3 (of HECT domain E3s family) and attached to the E3-bound substrate (C1). Direct transfer of ubiquitin from E2 to substrate is catalyzed by E3 (of RING finger domain E3s family) (C2). Ubiquitin can be released from substrate by DUBs (D). Lys48 or Lys29 linked polyubiquitin chain is synthesized by multiple cycles of conjugation of ubiquitin (E) and serve as a signal for ATP-dependent recognition of substrate (F) and its degradation by 26S proteasome (G). Monoubiquitination (H) and polyubiquitination via Lys 63 (I) do not represent the degradation signal but play a role in several cellular processes (J). Recycling of ubiquitin is mediated by specific subunits of 19S regulatory complex that are DUBs. The substrate is degraded to short peptides, which can be used in proteosynthesis or presented as an antigen.

this region ensures specificity of protein substrates recognition (Ciechanover, 1998). HECT-domain E3s firstly catalyze the transfer of ubiquitin from E2s to their active site and secondly the transfer of this ubiquitin from Cys residue in their active site to Lys residue in the substrate.

The next two families of E3s belong to the superfamily of RING finger motif-containing E3s that catalyze the direct transfer of ubiquitin moiety from E2s to Lys residue of the substrate. The third family of E3 enzymes is a high-molecular-weight complex, cyclosome (Sudakin et al, 1995) also called APC (anaphase promoting complex) (King et al, 1995). The substrates of these E3s are cell-cycle regulatory proteins, such as mitotic cyclins, some anaphase inhibitors and spindle-associated proteins, which are degraded at the end of mitosis. All of these substrates contain a nine amino acid degenerated motif called the destruction box. This complex is inactive during the interphase of cell cycle, but at the end of mitosis it is converted to the active form by phosphorylation mediated by the cyclin B/cyclin-dependent kinase 1 (Cdk1) complex MPF (M-phase promoting factor) (Lahav-Baratz et al, 1995).

The fourth family of E3s contain a different type of multisubunit ubiquitin ligase that is involved in the proteolysis of some other cell-cycle regulatory proteins, such as the Sic1 Cdk inhibitor or certain G1 cyclins. Phosphorylation of the substrate is necessary for its recognition by the ubiquitin ligase complex. These complexes, called PULCs (phosphoprotein-ubiquitin ligase complexes) or SCFs (Skp1-cullin-F-box protein ligase complexes) share some common subunits, but also contain subunits, which are specific for certain substrates (Hershko and Ciechanover, 1998).

Signals for ubiquitination

Specific protein motifs for ubiquitination and degradation are recognized by different types (families) of E3 enzymes. These motifs can be divided in two types, primary and secondary. The difference consists in the post-translational modifications of secondary motifs. Primary motifs are signals inherent in primary protein structure and do not require other modifications to provide a signal for degradation of substrates. However, the presence of these primary motifs does not necessarily lead to degradation because they can be hidden. If proteins are damaged or dissociated to subunits, the motifs are exposed and the proteins are degraded (Ciechanover, 1998).

The best characterized primary signal is the N-end rule system (Varshavsky, 1996). In this system, the degradation of protein is based on the recognition of its N-terminal amino acid residue by the specific E3 enzymes, N-end rule E3 (first family of E3). The character of N-terminal residues of protein determines its destiny and half-life. The N-terminal residues are divided to a few groups, primary, secondary and tertiary, destabilizing N-terminal residues and stabilizing N-terminal residues. Furthermore, the primary destabilizing N-terminal residues are divided in two types in the yeast (type 1 includes Arg, Lys and His; type 2 includes Leu, Phe, Tyr, Ile and Trp) or to three types in the mammals (types 1 and 2 are the same as in the yeast and type 3 contains Ala, Ser and Thr). Secondary destabilizing N-terminal residues are Asp and Glu in both yeast and mammals, but Cys only in mammals. Tertiary destabilizing N-terminal residues are Asn and Gln. The stabilizing N-terminal residues in both yeast and mammals are Met, Gly, Val and Pro. The stabilizing N-terminal residues specific for the yeast are Ala, Ser, Thr and Cys. The proteins with the stabilizing N-terminal residues are metabolically stable (their half-life is more than 20 h), in contrast to the proteins with the destabilizing N-terminal residues (the half-life of these proteins is less than 1 h) (Varshavsky, 1996). The tertiary destabilizing N-terminal residues are converted to secondary destabilizing residues by N-terminal aminohydrolase (Nt-amidase). In mammals, this deamination step is mediated by two enzymes. The first enzyme is Nt^N-amidase for Asn (N) residue and the second enzyme is Nt^Q-amidase for Gln (Q) residue, but in the yeast there is only one Nt-amidase for both residues (Hershko et al, 2000). The next step is a conversion of secondary destabilizing residues to Arg (R), one of the primary destabilizing residues, by Arg-RNA protein transferase (R-transferase). N-end rule E3 enzymes have two binding sites for N-terminal residues. The type I site is specific for the first type of primary destabilizing residues, basic residues, Arg, Lys and His. The second site, type II, is specific for the type 2 primary destabilizing hydrophobic residues – Leu, Phe, Tyr, Ile and Trp. This mechanism of recognition of the N-terminal residues of proteins is highly conserved, and was found in all eukaryotes, and even in prokaryotes, which do not produce ubiquitin (Hershko et al, 2000). A destruction signal, called N-degron, consists of a substrate's destabilizing N-terminal amino acid residue and an internal Lys residue of this substrate. The selection of internal Lys residue, which serves as a ubiquitination-site, is a stochastic process among several spherically suitable Lys residues of the substrate (Varshavsky, 1996).

Secondary motifs provide signal for degradation only if they are modified. Many rapidly degraded proteins are marked by phosphorylation and this post-translational modification serves as a signal for ubiquitination. In these proteins, PEST elements (proline (P), glutamic acid (E), serine (S), threonine (T)) were found. They consist of sequences enriched in Pro, Glu, Ser and Thr residues, which serve as phosphorylation sites (Rogers et al, 1986; Rechsteiner and Rogers, 1996). PEST sequences are required for phosphorylation and degradation of the yeast G1 cyclin Cln3 (Yaglom et al, 1995) and Gcn4 (Kornitzer et al, 1994) transcriptional activator. However, other proteins such as the mammalian G1 cyclin D1 are targeted by phosphorylation, too. The residues for phosphorylation are not in the PEST sequences. The signals that tag substrates for degradation such as a phosphorylation signal, have various functions. The phosphorylation is required for degradation of some substrates, but it also serves as a protection signal for other substrates. The phosphorylation of c-Mos protooncogene and the multiple phospohorylation of c-Fos and c-Jun protooncogenes by MAP kinases suppress their degradation by the ubiquitin-proteasome pathway (Glickman and Ciechanover, 2002).

Another secondary motif inheres in the substrate of E3 enzymes from HECT domain family, p53 as previously mentioned. Tumor suppressor p53 is bound to its E3s (E6-AP) via an ancillary protein, E6 oncoprotein. The modification of this motif in p53 by linkage with an ancillary protein is necessary for the recognition by E3 enzymes (Scheffner et al, 1993). An important signal for degradation of cell-cycle regulatory proteins, such as mitotic cyclins, some anaphase inhibitors and spindle-associated proteins, is the destruction box. It is a 9-amino acid motif with the following general structure: $R_1\,(A/T)_2\,(A)_3\,L_4\,(G)_5\,X_6\,(I/V)_7\,(G/T)_8\,(N)_9$. This sequence was obtained from nearly 40 B-type and A-type cyclins from various organisms and it is usually located approximately 40–50 amino acid residues apart from the N-terminus of substrates. The amino acid residues shown in parentheses occur in more than 50 % of known sequences of destruction boxes. Thus, only the first and the fourth amino acid residues, Arg in position 1 (R_1) and Leu in position 4 (L_4), are invariable. The destruction box is recognized by an E_3 enzyme called cyclosome or APC (Hershko and Ciechanover, 1998).

Proteasome

The second step of ubiquitin-proteasome pathway is mediated by the 26S proteasome, a remarkable protein complex, capable of recognizing and degradation of tagged proteins (Coux et al, 1996). After proteolysis of the substrate, short peptides and reusable ubiquitin are released. The release of ubiquitin from the substrate is catalyzed by deubiquitinating enzymes. The 26S proteasome is composed of two types of complexes, the 20S core complex and 19S regulatory complexes which are located on both sites of the 20S complex (Groll et al, 1997). The 20S core

complex is arranged as a stack of four heptameric rings, which are organized in the general structure of αββα. Each of these rings are composed of seven distinct subunits and so the general structure of 20S complex is $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$. The crystal structure has shown that the ß rings are necessary for the catalytic activity of proteasome because some of the ß subunits contain three catalytic sites, the trypsin-, the chymotrypsin- and the post-glutamyl peptidyl hydrolytic-like sites. The crystal structure has also shed light on the role of α rings. Although the α rings are catalytically inactive, they play an essential role in stabilizing the structure of ß rings and they are also involved in binding of 19S regulatory complexes. 20S complex contains a large interior chamber that contains the proteolytic active sites. In the *Thermoplasma acidophilum* proteasome is this chamber accessible only through the pores at the ends of the cylinder (Hershko and Ciechanover, 1998). It is surprising that these pores do not exist in the yeast 20S core complex and thereby the entry to the interior chamber is not possible, as N-terminal domains of some α subunits protrude and block the entry of substrates. Entry is possible only after a substantial rearrangement of the complex. This rearrangement is ATP-dependent and it can occur after association with the 19S regulatory complex, which has ATPase activity. Each 19S regulatory complex is composed of 15–20 subunits. Some of the proximal subunits of 19S complex are ATPases which serve probably to promote ATP-dependent substrate unfolding and translocation. Another subunit of complex is a deubiquitinating enzyme, which provides the release of ubiquitin from substrates (Pickart, 2000). Besides, the specific subunits of 19S regulatory complex probably mediate the recognition of substrate by the interaction with polyubiquitin chains. The polyubiquitin chain is made of four ubiquitins – the shortest chain that binds well to these polyubiquitin-binding subunits (Thrower et al, 2000). Thus, the signal for degradation of substrates by proteasome is only the chain of at least four ubiquitins, but not a single ubiquitin. Some of these polyubiquitin-binding subunits have been described in human (S5a), yeast (RPN10, MCB1) and plants (MBP1). PA28 (REG or 11S) is an additional complex that associates with 20S core complex and the formation of complex PA28-20S-PA28 is ATP-independent, in contrast to the formation of complex 19S-20S-19S which is ATP-dependent (Song et al, 1997). The PA28-20S-PA28 complex is not able to degrade a tagged protein but it digests only oligopeptides for antigen presentation.

The regulatory role of ubiquitin vs the regulation of ubiquitin

Deubiquitinating enzymes (DUBs) play an important role in the ubiquitin system. They are divided into two families. The first family is represented by ubiquitin C-terminal hydrolases (UCHs) which are involved in the release of ubiquitin from small molecules and peptides. Ubiquitin-specific proteases (UBPs, also called isopeptidases) belong to the second family of deubiquitinating enzymes catalyzing the release of ubiquitin from the polyubiquitin precursor chains and also serving as inhibitors of 26S proteasome because they are able to release ubiquitin from the tagged proteins (Wilkinson, 1997). DUBs can accelerate proteolysis by increasing the ubiquitin pool. This is achieved by the release of ubiquitin from its precursors and from the terminal product of ubiquitin-mediated proteolysis. DUBs can also inhibit degradation of proteins by removing ubiquitin form tagged proteins. Some of DUBs affect the diverse processes in the cell, including biogenesis of ribosomes, regulation of cell proliferation, gene silencing, long-term memory, development and signal transduction (Baek, 2003). Ubiquitination is a reversible modification similar to phosphorylation. Phosphorylation by kinases and dephosphorylation by phosphatases regulate the homeostasis of a number of proteins in the cell. Ubiquitination may also serve as a regulatator of homeostasis of some proteins by a cascade of three enzymes (E1, E2 and E3 enzymes) and deubiquitination by DUBs.

The ubiquitin system plays a role in many processes in the cell, some of them are carried out by the proteolysis and proteasome-dependent functions, which are described above. For the degradation of protein substrate it is necessary that the Lys48-linked polyubiquitin chain (or Lys29) contains at least four ubiquitins. Other processes are proteasome-independent and they include the attachment of a single ubiquitin (monoubiquitination) and polyubiquitination via Lys63 (Schnell and Hicke, 2003). Lys63-linked chains are involved in DNA repair (Spence et al, 1995), ribosome function, mitochondrial DNA inheritance, stress response and endocytosis. Monoubiquitination is necessary for the endocytic pathway, apoptosis (Lee and Peter, 2003), DNA repair, and it also plays a role in the regulation of activity of transcription factors and histones. It is a specific type of post-translational modification of proteins, similar to phosphorylation, and it may induce conformational changes in proteins resulting in regulation of activity of these targeted proteins (Riezman, 2002).

Transcription that is mediated by the RNA polymerase II (Pol II) in eukaryotic cells is partially regulated by the ubiquitin system. This regulation involves ubiquitin-dependent degradation of specific Pol II transcription factors and non-proteolytic mechanisms (monoubiquitination) (Conaway et al, 2002). Several of these transcription factors are activated by ubiquitin-proteasome system and their inactive precursors are proteolytically processed to active forms. The proteins involved in various phases of the programmed cell death are regulated by monoubiquitination, as well as polyubiquitination (Lee and Peter, 2003). The yeast E2 enzyme called Rad6 is linked to DNA repair. It is the first enzyme of the ubiquitin pathway with specific non-proteolytic function discovered. Mutagen chemicals and the ultraviolet light can create gaps in one of the two synthesized strands. Rad6 protein uses an undamaged strand as a template for post-replicative synthesis of DNA to fill the gaps (Pickart, 2002). These examples show how important and crucial may be the ubiquitin system and its defects in carcinogenesis and other pathological processes.

The ubiquitin system is one of the most interesting research fields in molecular biomedicine. The importance is based on its ubiquitous presence in every eukaryotic cell, the conserved char-

acter during the evolution and the regulatory role that affects most cellular processes. Although the significance of ubiquitination in cellular physiology and pathophysiology is undoubted, the use in clinical medicine is still at beginning. Nevertheless, as future research will bring new insights into the regulatory role of ubiquitination, the biomedical applications of the results will gain more importance.

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Received January 31, 2005.
Accepted February 20, 2005.