UBİKULİTİN SİSTEMİNİN YAPI VE FONKSİYONLARI

STRUCTURE AND FUNCTIONS OF UBIQUITIN SYSTEM

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Özet

Ubikuitin, ökaryotik hedef proteinlerin bir çoğuna ATP eşliğinde kovalent şekilde bağlanarak çeşitli regülatör prosesleri yönlendiren yüksekçe korunmuş bir proteindir. Bu makalede, ubikuitin sistemi ve hücredeki fonksiyonu literatür ışığında araştırıldı.

Anahtar kelimeler: *Ubikulitin, Ubikulitin aktive edici enzim.*

Summary

Ubiquitin (Ub) is a highly conserved protein involved in several important regulatory processes through its ATP-dependent, covalent ligation to a variety of eukaryotic target proteins. In this article, I have searched the literature to explain the ubiquitin system and its functions within the cell.

Key words: *Ubiquitin, Ubiquitin-activating enzyme.*

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Introduction

The degradation of proteins within cells depends upon the constant supply of metabolic energy (1). Since proteolysis per se is an exergonic process, it has been generally assumed that energy is required for selectivity or control. The mechanisms of energy-dependent protein breakdown have therefore been the subject of intensive investigation. That the conjugation of Ub with proteins may play an important role in the energy dependent degradation of intracelllar proteins. Ub is a small (Mr 8500) heat stable polypeptide of universal occurrence in all eukaryotic cells. It is a relatively abundant protein with estimates of intracelluler concentrations in the micromolar range. Roughly 50 % of the total intracelluler Ub pool is conjugated to various proteins (2), some of which are intermediates in Ub-mediated protein breakdown. The most thoroughly characterized conjugation event occurs in the Ub-dependent degradative pathway (3). Indeed, Ub was localized to the cytoplasm, nucleus, microvilli, autophagic vacuoles, and lysosomes (4). In addition, Ub-protein conjugates have also been identified associated with the cytoskeleton (5), histones and arthrin (6) the cytoplasm, lysosomes (7) and mitochondrial/endoplasmic reticulum.Perhaps the most remerkable feature of this protein is the extraordinary degree of sequence homology from species as diverse as yeast and man. Such a high degree of sequence homology probably reflects selective pressure arising from involvement of Ub in more than one critical life process.

Ubiquitin Structure

Ub was discovered by Goldstein(8) 1975 during the characterisation of putative calf thymus peptide hormones. Its primary structure was determined by Schlesinger(9) That is shown in figure 1. The sequence of Ub is identical in organisms as diverse as cattle (9), man (10), trout (11), toad (12), and insects (13). Ub is present in all eukaryotic cell types and is highly conserved, differing by only three amino acids from yeast to man (14). The three-dimensional structure of Ub shows it to be very compact with approximately 87 % of the polypeptide chain involved in hydrogen-bonding (15). Secondary structural elements of the molecule include five strands of β-configuration, three and one-half turns of α-helix, a short section of 3.10 helix and seven reverse turns. The last four residues at the carboxyl terminus of Ub are unstructured and they protrude from the overall globular domain to allow reaction with other proteins in the cell. These structural features account for the previously observed high resistance of Ub to heat denaturation, or to treatments with acid, alkali or denaturants (16). There are no cysteine or tryptophan residues in the molecule. Mild oxidation of the single amino-terminal methionine residue with hydrogen peroxide does not affect the activity of the protein (17). The polypeptide chain comprises 76 amino acids with a molecular weight of 8500, which makes it one of the smallest proteins in most cells.

Ubiquitin Genes

The gene of Ub, recently elucidated in various organisms by several groups of investigators, has a

Figure 1. *Amino Acid Sequence of Ubiquitin From Higher Eukaryotes.*

Met-Gln-Ile-Phe-Val[5]-Lys-Thr-Leu-Thr-Gly[10]-Lys-Thr-Ile-Thr-Leu[15]-Glu-Val-Glu-Pro-Ser[20]-Asp-Thr-Ile-Glu-Asn[25]-Val-Lys-Ala-Lys-Ile[30]-Gln-Asp-Lys-Glu-Gly[35]-Ile-Pro-Pro-Asp-Gln[40]-Gln-Arg-Leu-Ile-Phe[45]-Ala-Gly-Lys-Gln-Leu[50]-Glu-Asp-Gly-Arg-Thr[55]-Leu-Ser-Asp-Tyr-Asn[60]-Ile-Gln-Lys-Glu-Ser[65]-Thr-Leu-His-Leu-Val[70]-Leu-Arg-Leu-Arg-Gly[75]-Gly

unique organization. The genes responsible for encoding ubiquitin can be divided into three classes in eukaryotes. Primary structures are highly conserved between species in all of these classes (18). Class I and II Ub genes encode fusion proteins where ubiquitin is attached directly at its carboxyl terminus with ribosomal proteins ranging in size between 52 and 81 amino acid residues (19,20). Class III genes code for poly-Ub proteins where several Ub moieties are lined up head to tail. These genes contain heat shock promoter regions. Thus, they are expressed at a higher level during cell stress (21). In yeast, four different genes, termed UBI1, UBI2, UBI3 and UBI 4, have been found to code four Ub. UBI1, UBI2, UBI3 all code for ubiquitin fused to carboxyl-terminal extension sequences. UBI1 and UBI2 each contain two introns that differ only in size. Thus the fusion proteins encoded by both genes are 128 residues long and consist of Ub attached to an unrelated 52 residue sequence (14). The carboxyl-terminal 52 residues are predominantly basic, with lysine and arginine making a 31% contribution. The intact fusion protein or its 52 residue tail could, therefour, function as a nucleic acid binding protein and, in this way, it was initially suggested that it might act as a carrier to transport ubiquitin to the nucleus for its known conjugation to the nucleus for its known conjugation to histones (22):a role in binding to RNA in the ribisome now appears to be more likely. The amino acid sequence of the 76 residues fused to the carboxyl terminus of ubiquitin in UBI3 bears little similarity to that of the 52 residue tail in UBI1 and UBI2 and has been shown to function as a ribosomal protein (18,19,23): the presence of Ub at its amino terminus may facilitate the incorporation of the ribosomal protein into the ribosome (20). The UBI4 gene, which belongs to class III, encodes a 43 kDa product that is composed of five identical Ub repeats joined head-to-tail with an additional carboxyl-terminal single amino acid; the latter may function in prohibiting ligation of the unprocessed precursor to cellular proteins. There are three human Ub genes designated UBIA, UBIB and UBIC. UBIA is a class II gene and encodes Ub with an 80 amino acid residue tail. Although the tail contains large numbers of basic amino acids, as in the yeast UBI1-UBI3 genes, the amino acid sequence is quite different. UBIB and UBIC are class III genes with heat shock promoters and encode poly-Ub sequences composed of three and nine Ubs, respectively.

Ubiquitin-Protein Conjugates

Protein breakdown is responsible for essential cellular functions such as the modulation of the levels of key enzymes and regulatory proteins and removal of abnormal proteins that arise by biosynthetic errors or postsynthetic damages. The Ub that is found in all eukaryotic cells can be either free or covalently linked to other cellular proteins. A majority of the cellular Ub-protein conjugates are derived from the formation of an isopeptide bond between the carboxyl-terminal carboxyl group of Ub and the ε-amino group of a lysine residue in an acceptor protein. Proteins may have Ub added singly or multiply. In yeast cells, Varshavsky and co-workers (24) have shown that rapidly degraded derivatives of β-galactosidase are converted to Ub conjugates at a higher rate than slowly degraded derivatives. Proteins to which a single Ub is attached include histones H2A and H2B, the lymphocyte homing receptor and growth hormone receptor. In cultured animal cells, about 10% of H2A (25) and 1-1.5 % of H2B (26) are in the form of their conjugates with Ub. The significance of these mono-Ub conjugates is not clear, since these proteins are not short-lived in vivo. As noted earlier, Ub is also found linked to the amino terminus of certain proteins which are linear rather than branched in form. In these structures Ub may serve to enhance the efficiency of expression of the non-Ub component of fusion. Target protein multi-Ub involving lysine 48 of Ub is known to occur during protein degradation in the ATP and Ub-dependent proteolytic pathway. Multiubiquitination, with the carboxyl terminus of one Ub linked to Lys-48 on another molecule, targets proteins for degradation (27). Recently, it has been suggested that Lys-48 may not be the only lysine residue in Ub that can serve as an Ub acceptor site (28).

Conjugating enzymes

Ub functions in the cell through its reversible conjugation to acceptor proteins. The pathway was first studied in reticulocyte lysates, where it was shown that conjugation is catalysed by a multi-enzyme system. A three-step mechanism for Ub-protein conjugate formation has been proposed (29).

$$2ATP+2Ub+E1 \xrightarrow{Mg^{2+}} \text{AMP-Ub-S-Ub} + 2PPi+AMP \quad (1)$$

$$E1\text{-S-Ub}+E2 \xrightarrow{E3} E2\text{-S-Ub}+E1 \quad (2)$$

$$E2\text{-S-Ub}+RNH_2 \rightarrow E2+\text{Ub-NHR} \quad (3)$$

After activation to a thiol ester by Ub-activating enzyme (E1) (equation 1), Ub is transferred to thiol groups of Ub-carrier enzymes (E2s) (equation 2). One or more of the E2-Ub thiol esters then denates Ub to a protein amino group, in a reaction catalyzed by Ub-protein ligase (E3) (equation 3).There are two active sites within the E1 molecule, accommodating two Ub moieties at a time with a new Ub forming an adenylate intermediate as the previous one is transferred to the thiol site (30,31). The role of AMP-Ub is to provide the activated Ub necessary for thiol ester formation. Binding of AMP-Ub to E1 serves to position Ub correctly for reaction with the E1 thiol group. Hence, Ub activation is ATP-dependent and requires an initial adenylation of the carboxyl-terminal glycine residue of Ub. Isotope exchange between ATP and PPi by proposing formation of an Ub-adenylate intermediate. A possible solution to the problem of a second intermediate to account for ATP:AMP exchange is suggested by the observation that activating enzyme forms a covalent bond to Ub having properties consistent with a thiol ester between the COOH-terminal glycine of the polypeptide an a sulfhydryl residue of the enzyme (32). Genes encoding E1 have been cloned from yeast, wheat and man. A gene coding for the E1 is located on the x chromosome, albeit the evidence is indirect. E1 may be required for the progression of the S phase and also for the onset of chromosome condensation. The enzyme has also been purified from a variety of sources and appears to have a molecular mass of about 105 kDa, both under denaturing and non-denaturing conditions, suggesting that E1 is monomeric (33,34,35). E1 is also abundant within the cytoplasm where it has been found in association with the cytoplasmic surface of endosomal/lysosomal vacuoles and within the mitochondria (36,37). In our doctoral thesis, we purified E1 enzyme from human placenta, which was not shown previously in the literature, and we showed its localisation in the placenta immunocytochemically (38). In another study, we investigated the kinetic properties of E1 and found that ethilmaleimid, EDTA inhibited the enzyme of which optimum pH was 7 and optimum temperature was 35 °C (39). Immunocytochemical studies using antibodies raised against purified E1 show that E1 is highly concentrated in both yeast and mammalian cell nuclei. Although the roles of E1 in the nucleus are not known, it has been suggested that it may be involved in ubiquitination of specific nuclear proteins such as histones or degradation of other nuclear proteins (40). In the second step of conjugation, E2s mediate the transfer of Ub from E1 to the protein substrate, with or without the participation of Ub-protein ligases (E3). The five Ub carrier proteins are relatively low molecular weight proteins that share the ability to form a Ub thiol ester, using the E1-Ub thiol ester as Ub donor. The proteins have been shown to generate thiol esters with ubiquitin in the presence of E1 (41). A number of genes coding for E2 proteins have been cloned and sequenced from plants, yeast and mammals (42). Thus far, at least seven genes that encode different E2s have been identified in yeast. A number of E2s have been purified from rabbit reticulocytes (41-44). and human placenta (45). Analysis of some of the E2 genes in yeast showed that all of the proteins share a core domain of approximately 150 amino acid residues with 35% sequence homology. The active site cysteine, required for formation of thiol esters with Ub, is localised on this domain (18). In view of the possibility of different functions for the individual E2s, it was of interest to separate these components and characterize them with respect to specificity in reaction with simple amines, ability to form protein conjugates, and role in protein breakdown. In addition to this, some comparisons have been made between E2s purified from different species. Western blot analyses showed that rabbit reticulocyte $E2_{14kDa}$, $E2_{17kDa}$ and $E2_{25kDa}$ reacted with antibody raised against the yeast 20kDa E2 (also known as RAD6) (46). Human placenta lacks the $E2_{14kDa}$ and $E2_{20kDa}$ enzymes. However, placental $E2_{17kDa}$ showed 69% primary sequence homology with yeast $E2_{20kDa}$ (45). In the third step, Ub is transferred from the E2-Ub thiol ester to an internal Lys residue of the target protein. For some targets, this latter step requires a third enzyme, E3 (47). Since E3 preferentially recognizes proteins with unblocked amino termini, these results suggest that E3 may not participate in the Ub-dependent destruction of acetylated proteins. Two forms of E3, α and β, have been purified from reticulocytes. Both have molecular masses of 350-kDa and 180-kDa subunits (48). E3α has been shown to contain specific sites for the binding of protein substrates prior to their ubiquitination. The selection of proteins for degradation by the Ub system is effected by the specificity of binding to E3 and it has been

demonstrated that the nature of the amino-terminal residue of a protein is important for this (49). However, little is known about the mechanism of the chemical step catalyzed by the ligase: there are no known inhibitors of the enzyme. Additionally, the products that are formed in the conjugation reaction have complex and heterogeneous structures. There is, therefore, still only a limited understanding of the functioning of E3 molecules.

Roles of ubiquitin

Protein degradation occurs in all types of cells and, in addition to roles in anormal protein turnover, degradation systems perform a number of related functions such as the breakdown of denatured or foreign proteins. Although rates of protein turnover are very heterogeneous, proteins can be roughly divided into two groups, short-lived and long-lived. Regulatory proteins or enzymes have fast turnover rates, so that their levels can be rapidly changed in response to appropriate stimuli: in some cases the rates of degradation of regulatory proteins are controlled with high temporal precision, for example the cyclins whose levels are controlled at certain stages of the cell cycle. The degradation arm of protein turnover is divisible into two general mechanism, lysosomal and non-lysosomal (35). Ub is a conserved eukaryotic protein which is found in the cytoplasm, in the nucleus and on the cell surface. Cytoplasmic Ub is known to function in proteolysis, whereas the precise role of nuclear Ub is still undefined. In animal chromatin, a fraction of histones H2A and H2B have Ub covalently attached. Ub is also present in vivo as a specific isopeptide derivative of lysine 117 of histone H2A (50). Ubiquitinated nucleosomes are preferentially located within actively transcribed chromosomal regions in Drosophila. In addition, the levels of ubiquitinated histones have been shown to vary with the position in the cell cycle and with physiological stress. Although it was suggested some time ago that ubiquitinated histones could control mitosis or that Ub could play a role in histone or nucleosome degradation (51), precisely how this modification of histones affects transcription or mitotic processes is still not known. Moreover, the formation of ubiquitinated histones as well as the synthesis of Ub are linked neither to DNA nor to histone synthesis. In contrast, however, the structures of histone-Ub conjugates are known in detail. It has also been shown that an actin of insect flight muscle(6), lymphocyte homing receptor(52), platelet-derived growth factor receptor (53), and growth hormone receptor(54) are ubiquitinated. The function or functions of ubiquitination of these proteins is not clear.

References

1. Hershko, A., Tomkins, G.M. (1971):Studies on the degradation of tyrosine aminotrasferase in hepatoma cells in culture. Influence of the composition of the medium and adenosine triphosphate dependence. J Biol Chem 246(3):710-714.
2. Carlson, N., Rogers, S., and Rechsteiner, M. (1987): Microinjection of ubiquitin: Changes in protein degradation in HeLa cells subjected to heat-shock. J Cell Biol 104:547-555.
3. Reichsteiner, M., ed. (1988):Ubiquitin. Plenum, New York.
4. Bond, U., Schlesinger, M.J. (1986):The chicken ubiquitin gene contains a heat shock promoter and expresses an unstable mRNA in heat-shocked cells. Mol Cell Biol 6(12):4602-4610.
5. Murti, K.G., Smith, H.T., Fried, V.A. (1988): Ubiquitin is a component of the microtubule network. Proc Natl Acad Sci 85:3019-3023.
6. Ball, E., Karlik, C., et al. (1987): Arthrin, a myofibrillary protein of insect flight muscle, is an actin-ubiquitin conjugate. Cell 51:221-226.
7. Laszlo, L., Doherty, F., Osborn, N.U., Mayer, R.J. (1990): Ubiquitinated protein conjugates are specifically enriched in the lysosomal system of fibroblasts. FEBS 261:365-368.
8. Goldstein, G., Scheid, M., Hammerling, U., Boyse, E.A., et al. (1975): Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proc Natl Acad Sci 72:11-15.
9. Schlesinger, D.H., Goldstein, G., and Niall, H.D. (1975): The complete amino acid sequence of ubiquitin. An adenylate cyclase stimulating polypeptide probably universal in living cells. Biochemistry 14:2214-2218.
10. Schlesinger, D.H., Goldstein, G. (1975):Molecular conservation of 74 amino acid sequence of ubiquitin between cattle and man. Nature 255(5507):42304.
11. Watson, D.C., Levy, W.B., Dixon, G.H. (1978):Free ubiquitin is a non-histone protein of trout testis chromatin. Nature 276(5684):196-198.
12. Dworkin, R.E., Shrutkowski, A., Dworkin, M.B. (1984):Multiple ubiquitin mRNAs during xenopus laevis development contain tandem repeats of the 76 amino acid coding sequence. Cell 39:321-325.
13. Gavilanes, J.G., Gonzalez B.G., Perez, C.R., Rodriguez, R. (1982): Isolation, characterization, and amino acid sequence of a ubiquitin-like

protein from insect eggs. J Biol Chem 257(17):10267-10270.
14. Ozkaynak, E., Finly, D., Solomon, M., and Varshavsky, A. (1987): The yeast ubiquitin genes. EMBO J 6:1429-1439.
15. Vijay-Kumar, S., Bugg, C., Wilkinson, K., and Cook, W. (1985): Three-dimensional structure of ubiquitin at 2.8 A resolution. Proc Natl Acad Sci 82:3582-3587.
16. Ciechanover, A., Hod, Y., Hershko, A. (1978):A heat-stable polypeptide component of an ATP dependent proteolytic system from reticulocytes. Biochem Biopsy Res Com 81:1100-1105.
17. Breslow, E., Chauhan, Y., Daniel, R., and Tote, S. (1986): Role of methionine in ubiquitin conformation and activity. Biochem Biophys Res Common 138:437-442.
18. Jentsch, S., Seufert, W., and Hauser, H. (1991): Genetic analysis of the ubiquitin system. Biochem Biophys Acta 1089(2):127-139.
19. Redman, K., and Rechsteiner, M. (1989): Identification of the long ubiquitin extension as ribosomal protein S27a. Nature 338:438-441.
20. Finley, D., Bartel, B., and Varshavsky, A. (1989): The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. Nature 338:394-401.
21. Finley, D., Ozkaynak, E., and Varshavsky, A. (1987): The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation and other stresses. Cell 48:1035-1046.
22. Baker, R., and Board, G. (1991): The human ubiquitin-52 amino acid fusion protein gene shares several structural features with mammalian ribosomal protein genes. Nucleic Acids Res 19(5):1035-1036.
23. Warner, J. (1989): A marriage of convenience or necessity? Nature 338:379.
24. Bachmair, A., Finley, D., Varshavsky, A. (1986):In vivo half-life of a protein is a function of its amino-terminal residue. Science 234(4773):179-186.
25. West, M.H., Bonner, W.M. (1980):Histone 2A, heteromorphous family of eight protein species. Biochem 19(14):3238-3234.
26. West, M.H., Bonner, W.M. (1980):Histone 2B, can be modified by the attachment of ubiquitin. Nuc Acid Res 8(20):4671-4680.
27. Chau, V., Tobias, J., Bachmair, A., et al. (1989): A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science 243:1576-1583.
28. Finley, D., and Chau, V. (1991): Ubiquitination. Annu Rev Cell Biol 7:25-69.
29. Hershko, A., Heller, H., Elias, S., Ciechanover, A. (1983): Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. J Biol Chem 258(13):8206-8214.
30. Wilkinson, K.D., and Mayer, A. (1986): Alcohol-induced conformational changes of ubiquitin. Arch Biochem Biophys 250:378-383.
31. Handley, P., Mueckler, M., Siegel, N., et al. (1991): Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme, E1. Proc Natl Acad Sci 88:258-262.
32. Ciechanover, A., Heller, H., Katz-Etzion, R., Hershko, A. (1981):Activation of the heat stable polypeptide of the ATP dependent proteolytic system. Proc Natl Acad Sci 78:761-765.
33. Hatfield, P., and Vierstra, R. (1989): Ubiquitin-dependent proteolytic pathway in wheat germ: isolation of multiple forms of ubiquitin-avtivating enzyme, E1. Biochemistry 28:735-742.
34. Hoefer, M., and Cook, J.C. (1991): Purification and partial characterisation of ubiquitin-activating enzyme from saccharomyces cerevisian. FEBS lett 289 (1):54-58.
35. 35.Hershko. A., and Ciechanover, A. (1992): The ubiquitin system for protein degradation. Annu Rev Biochem 61:761-778.
36. Schwartz, A., Trausch, J., Ciechanover, A., et al. (1992): Immunoelectron microscopic localization of the ubiquitin-activating enzyme E1 in HepG2 cells. Proc Natl Acad Sci 89:5542-5546.
37. Lenk, S., Dunn, W.ü Trausch, J., et al. (1992): Ubiquitin-activating enzyme, E1, is associated with maturation of autophagic vacuoles. J Cell Biol 118(2):301-308.
38. Kızıltunç, A. (1995):The purification of E1 from blood and placenta, antibody production and the tıssue dıstribution of E1. Doctoral thesis. Department of Biochemistry, Atatürk University,School of Medicine, Erzurum-TÜRKİYE.
39. Kızıltunç, A., Pirim, I., Şahin, YN. (1994):Obtaining of ubiquitın activating enzyme and kinetic features.T Clin J Med Sci 15:260-264.
40. Cook, J., and Chock, P. (1991): Immunocytochemical localization of ubiquitin-activating enzyme in the cell nucleus. Biochem Biophy Common 174(2):564-571.
41. Pickart, C., and Rose, I. (1985): Functional-heterogeneity of ubiquitin carrier proteins. J Biol Chem 260(3):1573-1581.

42. Cook, W., Jeffrey, L., Sullivan, M., and Vierstra, R. (1992): Three-dimensional structure of a ubiquitin-conjugating enzyme (E2). J Biol Chem 267(21):15116-15121.
43. Haas, A., Bright, P., and Jackson, V. (1988): Functional diversity among putative E2 isoenzyme in the mechanism of ubiquitin histone ligation. j Biol Chem 263(26):3268-3275.
44. Klemperer, N., Berleth, E., and Pickart, C. (1989): A novel arsenite-sensitive E2 of the ubiquitin pathway purification and properties. Biochemistry 28(14):6035-6041.
45. Schneider, R., Eckerskon, C., et al. (1990): The human ubiquitin carrier protein E2 (17kDa) is homologous to the yeast DNA repair gene, RAD6. EMBO J 9(5):1431-1435.
46. Berleth, E., and Pickart, C. (1990): Several mammalian ubiquitin E2s, but not E2 20kDa, are related to the 20kDa yeast E2, RAD6. Biochem Biophys Res Common 171(2):705-710.
47. Cecile, M.P., Eileen, M.K., et al. (1994): Subsrate properties of site-specific mutant ubiquitin protein (G76A) reveal unexpected mechanistic features of ubiquitin-activating enzyme (E1). The J Biol Chem 269(10):7115-7123.
48. Hershko, A., Heller, H., et al. (1986): The protein substrate binding site of the ubiquitin-protein ligase system. J biol Chem 261(26):1992-1999.
49. Reiss, Y., Kaim, D., and Hershko, A. (1988): Specificty of binding of NH2-terminal residue of proteins to ubiquitin-protein ligase use of amino acid derivatives to characterize specific binding sites. J Biol Chem 263(6):2693-2698.
50. Busch, H., Goldknoph, I.L. (1981): Ubiquitin-protein conjugates. Mol Cell Biochem 840:173-187.
51. Wu, R., Kohnk, W., and Bonner, W. (1981): Metabolism of ubiquitinated histones. J Biol Chem 256(11):5916-5921.
52. Siegelman, M., Bond, M., Gallatin, W., et al. (1986): Cell surface molecule associated with lymphocyte homing is a ubiquitinated branched-chain glycoprotein. Science 231:823-829.
53. Yarden, Y., Escobedo, J., Kuang, W., et al. (1986): Structure of the receptor for platelet derived growth factor helps define a family of closely related growth factor receptors. Nature 323:226-231.
54. Leung, D.W., Spencer, S., Cachianes, G., et al. (1987): Growth hormone receptor and serum binding protein: Purification, cloning and expression. Nature 330:537-543.