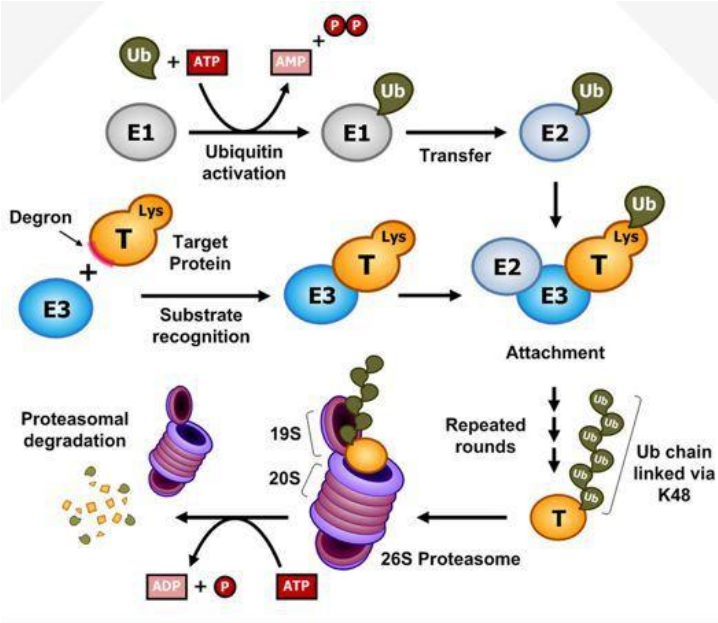
Proteolysis / Ubiquitin

Overview of Proteolysis



Proteolysis is the breakdown of proteins into smaller polypeptides or amino acids. Proteolysis is usually catalyzed by enzymes known as proteases, but can also occur through intramolecular digestion. Low pH or high temperature can also cause proteolysis without enzymolysis. In eukaryotes, there are two main mechanisms of protein degradation, lysosome and 26S proteasome. Lysosomes mainly deal with long-lived proteins in a non-selective manner, while proteasomes degrade abnormal or short-lived proteins in a strictly regulated manner.
Proteasome and lysosome are the two major proteolytic sites in eukaryotic cells. They degrade various proteins, but most of their substrates have very different properties, and there is not much overlap between the two systems. It is noteworthy that many studies have suggested the role of proteasomes in many pathological diseases, such as cancer and Alzheimer's disease.

Biological functions of Proteolysis

Post-translational proteolytic processing:

Limited proteolysis of polypeptide occurs in many proteins during or after translation in protein synthesis. This may involve removing N-terminal methionine, signaling peptides and/or converting inactive or nonfunctional proteins into active proteins. The precursors of the final functional form of protein are called proproteins.

Protein degradation:

Protein degradation can occur in cells or outside the cell. In digestive foods, digestive enzymes can be released into the environment for extracellular digestion, whereby proteolytic cleavage breaks down proteins into smaller peptides and amino acids that can be absorbed and used by organisms. Proteins in cells are also constantly broken down into amino acids. Intracellular degradation of this protein has many functions; it can remove damaged and abnormal proteins and prevent their accumulation, and it can also regulate cellular processes by removing unnecessary enzymes and regulating proteins. Amino acids can then be reused for protein synthesis.

Cellular regulation:

Proteolysis also involves the regulation of many cellular processes by activating or deactivating enzymes, transcription factors and receptors, such as cholesterol biosynthesis, or thrombin signaling mediated by protease-activated receptors. Some enzymes at important metabolic control points are completely regulated by their rate of synthesis and degradation. Other fast-degrading proteins include protein products of proto-oncogenes, which play an important role in the regulation of the cell growth.

Ubiquitin and ubiquitylation

- **Ubiquitin**

Ubiquitin is a small protein found in all eukaryotes (most eukaryotic cells). Ubiquitin is composed of 76 amino acids with a molecular weight of about 8500. Its main function is to mark the proteins that need to be decomposed, so that they are degraded by 26S proteasome. Some receptors on the 26S proteasome regulate the subunit to recognize the K48 and K11-linked polyubiquitinated proteins, and the 20S core subunit hydrolyzes the substrate under ATPase energy. Ubiquitin can also mark transmembrane proteins and participate in protein vesicle transport. Atypical ubiquitin chains play an important role in cell signaling, endocytosis, DNA damage

repair and regulation of NF-kB pathway. It is highly conserved in eukaryotes, and the similarity between human and yeast ubiquitin is 96%.

- **Ubiquitylation**

-

Ubiquitination refers to the process in which ubiquitin (a kind of low molecular weight protein) molecules classify the intracellular proteins under the action of a series of special enzymes, select the target protein molecules and modify the target protein specifically. These special enzymes include ubiquitin-activating enzymes, ubiquitin-conjugating enzymes, and ubiquitin ligases, known as E1s, E2s, and E3s. Ubiquitination plays an important role in protein localization, metabolism, function, regulation and degradation. At the same time, it also participates in cell cycle, proliferation, apoptosis, differentiation, metastasis, gene expression, transcriptional regulation, signal transduction, injury repair, inflammation and immune regulation of almost all life activities. Ubiquitination is closely related to tumours and cardiovascular diseases. The process consists of three main steps:
Activation: At the beginning of the ubiquitination cascade, E1 enzymes bind to ATP-Mg2+ and ubiquitin proteins and catalyze ubiquitin C-terminal adenylation. In the next step, the catalytic cysteine on the E1 enzyme attacks the ubiquitin-AMP complex through acyl substitution, producing thioester bonds and AMP leaving groups. Finally, E1-ubiquitin is transferred to E2 enzyme by transesterification of the protein complex, where E2 catalyzes cysteine to attack E1-ubiquitin on the back of the protein complex. However, the transport sulfation process is complex because both E1 and E2 enzymes form intermediate complexes, both of which undergo a series of conformational changes to bind to each other.
Conjugation: Ubiquitin-activating enzyme activates ubiquitin by covalently attaching molecules to cysteine residues at their active sites. The activated protein was then transferred to E2 cysteine. Once bound to ubiquitin, E2 molecules bind to one of several ubiquitin ligases or E3 proteins via a structurally conserved binding region. The E3 molecule binds to the target protein substrate and transfers the lysine residues that pervade the protein from E2 cysteine to the target protein. E2 enzymes are characterised by their highly conserved structure, known as the ubiquitin-conjugating catalytic (UBC) fold.
Ligation: E3 ubiquitin ligase catalyzes the final step of ubiquitination cascade. Most commonly, they produce isopeptide bonds between lysine of the target protein and glycine at the C-terminal of the protein. E3 enzyme acts as a substrate recognition module and interacts with E2 and substrate. Some E3 enzymes also activate E2 enzymes. Two main types of E3 are defined by two distinct sequence motifs, the RING domain and the HECT domain (homologous to the end of the E6-AP carboxyl group). These two ligases mediate the transfer of Ub in different ways, because Ub on E2 is transferred to active Cys in the HECT domain to form intermediates before

attaching to the substrate, while RING E3, which lacks active Cys residues, is used as a bridge to facilitate the direct transfer of Ub from E2 to the substrate.
Specific cells usually contain only a few types of E1 molecules, a greater diversity of E2s, and very large kinds of E3s. Therefore, the E3 molecule responsible for substrate identification and binding is the substrate specificity mechanism of proteasome degradation. Each type of E2 can be associated with many E3.

References:

1. Kimura Y, Tanaka K. Regulatory mechanisms involved in the control of ubiquitin homeostasis. Journal of Biochemistry. 2010, 147 (6): 793–8.
2. Schnell JD, Hicke L. Non-traditional functions of ubiquitin and ubiquitin-binding proteins. The Journal of Biological Chemistry. 2003, 278 (38): 35857–60.
3. Kathleen M. Sakamoto. Ubiquitin-dependent proteolysis: its role in human diseases and the design of therapeutic strategies (PDF). Molecular Genetics and Metabolism. 2002, 77 (1–2): 44–56.
4. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiological Reviews. 2002, 82 (2): 373–428.