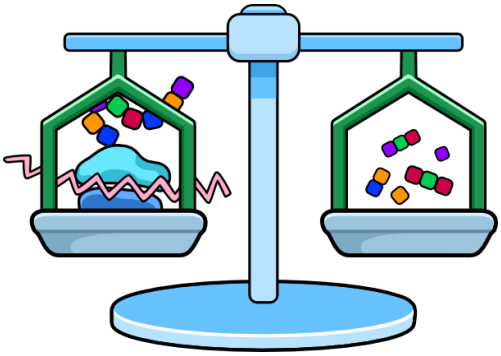
THE GREAT ENCYCLOPEDIA OF PROTEIN DEGRADATION

The Great Encyclopedia of Protein Degradation comprises **nine entries** that introduce the **mechanisms of protein degradation** and novel therapies centered around **targeted protein degradation**.

1. Protein homeostasis: the balance between synthesis and degradation

How proteins are made

Proteins are molecules responsible for performing many essential functions in the cell. Their formation begins with the transcription of DNA into messenger RNA (mRNA) in a process called transcription. The synthesized mRNA molecule then serves as a template for protein synthesis, termed translation, which takes place with the help of ribosomes. During translation, amino acids combine into a polypeptide chain, which forms a functional protein structure. A human cell can produce up to approximately 20,000 different proteins.



The balance between protein synthesis and degradation is essential for proper cellular function: left - protein synthesis is shown by the ribosome forming an amino acid chain based on mRNA (messenger RNA; matrix RNA); right - protein degradation depicted as a protein broken down into short amino acids chains.

How proteins are degraded

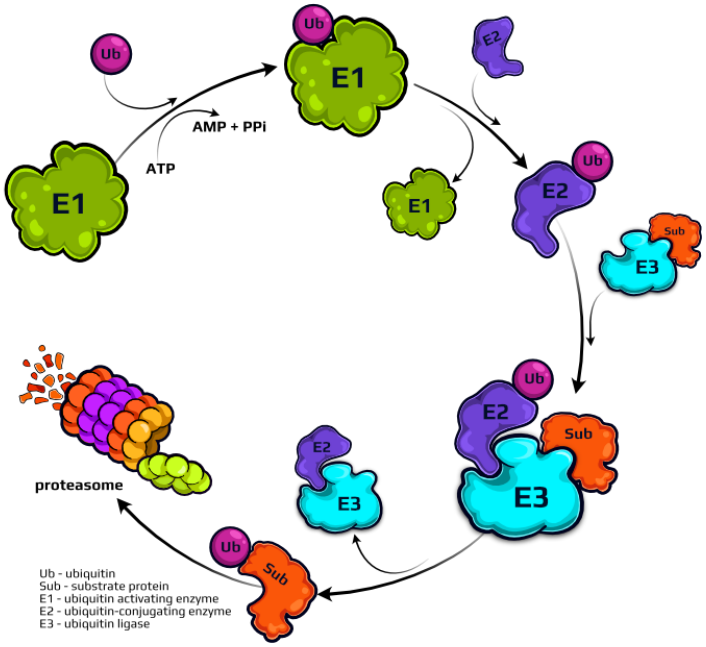
Maintaining protein homeostasis (equilibrium) requires efficient mechanisms for protein degradation. Every protein persists in a cell for a certain period, from seconds to minutes or even years. However, proteins can form incorrectly or contain the wrong amino acids, making their timely and accurate removal essential for proper cell function.
There are three main degradation pathways:

- autophagy: involves the engulfment of proteins by specialized intracellular vesicles called autophagosomes. These fuse with lysosomes (organelles surrounded by a lipid-protein membrane that acts like a digestive system), in which proteins are broken down into short amino acid chains or single amino acids, which can be reused as building blocks for new proteins;
- extracellular vesicles: cells can release small vesicles, such as exosomes, containing unwanted or excess proteins to the outside. The exosomes are then taken up by other cells, and the proteins inside them are degraded, ensuring their removal from the cellular environment;
- the ubiquitin-proteasome system (UPS): proteins targeted for degradation are labeled with a small protein called ubiquitin, which is recognized by the proteasome, a cellular recycling center, and broken down into short amino acid chains that can be reused by the cell.

2. Ubiquitin-proteasome system

In our game, we show the functioning of the ubiquitin-proteasome system (UPS). It involves a cascade of enzymes that includes E1, the ubiquitin-activating enzyme; E2, the ubiquitin-conjugating enzyme; and E3, the ubiquitin ligase, which work together to attach ubiquitin to the target protein. This process, called ubiquitination, results in proteins being recognized and then degraded by the proteasome. The proteasome acts as a cellular recycling center, breaking down ubiquitin-tagged proteins into short amino acid chains that can be reused by the cell to, for example, synthesize new proteins.

The UPS plays an important role in maintaining protein homeostasis by controlling protein levels and eliminating those that are unwanted. In this way, it regulates various molecular processes, such as the cell cycle, signal transduction, and DNA repair. Mutations of UPS components can lead to protein accumulation, disrupt cellular functions, and contribute to the development of various diseases, including cancer, Alzheimer's disease, and Parkinson's disease.

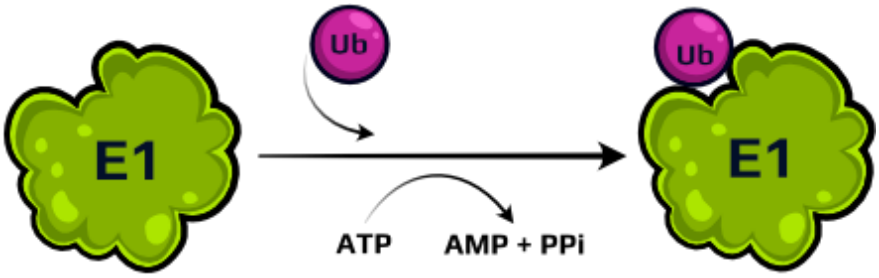


The UPS uses a cascade of enzymes to selectively label proteins with ubiquitin, allowing the proteasome to degrade them.

The UPS was discovered by Aaron Ciechanover, Avram Hershko, and Irwin Rose, and they were awarded the 2004 Nobel Prize in Chemistry for their groundbreaking research. Their work has revolutionized our understanding of protein degradation in cells, including the vital role of UPS and its impact on health.

3. E1 enzyme

Does ubiquitin magically appear on the E2 enzyme? Of course not! Although not mentioned in the game, it is necessary for the E1 enzyme to first activate ubiquitin. The mechanism begins with this enzyme binding both the ubiquitin molecule and adenosine 5'-triphosphate (ATP). This alters the shape of the E1 enzyme and allows it to form a high-energy bond between it and the ubiquitin molecule.

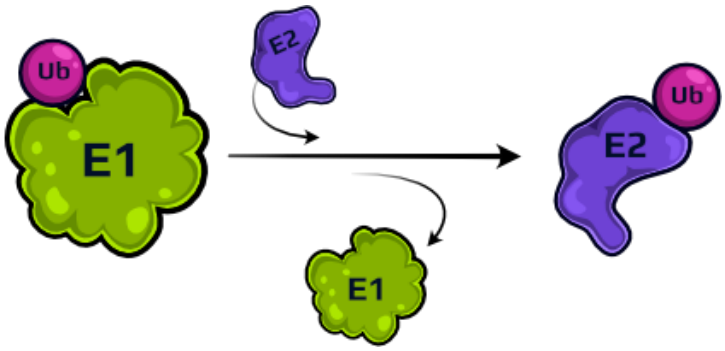


The E1 enzyme initiates the first step of ubiquitination by activating and binding ubiquitin.

There are only a few types of E1 enzymes in a cell, but mutations of these can lead to impaired activation of ubiquitin, resulting in reduced protein degradation. Such dysregulation of E1 enzymes has been linked to several diseases, such as infantile spinal muscular atrophy (XL-SMA), a serious neurodegenerative disorder linked to the X chromosome.

4. E2 enzyme

E2 ubiquitin-conjugating enzymes, of which there are 30 - 40 types, transfer activated ubiquitin from E1 enzymes to target proteins. The process proceeds as follows: the E2 enzyme forms a temporary high-energy bond with the activated ubiquitin, and then the E3 ubiquitin ligase steps in to mediate the transfer of ubiquitin to the target proteins.

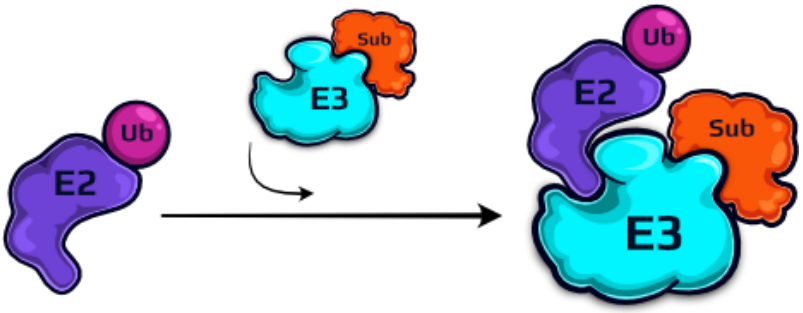


The second step in the ubiquitination process involves the transfer of ubiquitin from the E1 enzyme to the E2 enzyme.

Malfunctioning E2 enzymes, similar to E1 enzymes, cause various diseases, including cancer, X-chromosome-associated intellectual disability, and Fanconi anemia.

5. E3 enzyme

E3 ubiquitin ligases mediate the transfer of ubiquitin from E2 enzymes to target proteins, ensuring their specific and controlled ubiquitination. Humans are estimated to have approximately 600 - 1000 different types of E3 enzymes.



The third and final step of ubiquitination involves the recognition of the target protein by the E3 ubiquitin ligase, which brings the target protein into the vicinity of the E2 enzyme to attach the ubiquitin to it.

How do E3 ligases "know" which protein to select for degradation? Some recognize target proteins by identifying short amino acid sequences (the characteristic primary structure of a protein) on their surface, called degrons. Importantly, E3 ligases show specificity toward different degrons, enabling precise binding to their target proteins. E3 ligases can also detect misfolded proteins, which often expose their hydrophobic amino acids, and target them for ubiquitination, thus preventing aggregation (clumping) of harmful proteins. E3 ligases can also undergo auto-ubiquitination, which they use to regulate their activity, stability, and interactions with proteins.

Does the complex formed by the enzymes E2, E3, and the target protein always break down after each ubiquitination? In the cell, most of the processes are very dynamic, and the stability of the complex is affected by many factors, such as the strength of chemical bonds and pH. That is why some randomness can be observed in the game, for example, sometimes, after ubiquitination, the E3 ligase will remain bound to the target protein.

Abnormal functioning of E3 ligases contributes to various diseases. An example is a mutation of the parkin gene, encoding an E3 ligase, which is associated with the hereditary form of Parkinson's disease. In addition, defects in the E3 ligase MDM2 cause many cancers, such as breast, colon, and lung cancer, as well as soft tissue sarcomas.

6. Ubiquitin

Ubiquitin is a small, 76-amino-acid protein with a characteristic spatial structure. It is highly evolutionarily conserved and is found in all eukaryotes (organisms made of cells containing a nucleus), hence its name from the word “ubiquitous”. The attachment of ubiquitin to a target protein, or ubiquitination, is a type of post-translational modification, meaning that it is a form of chemical change that occurs in proteins after their synthesis. The attachment of just one ubiquitin is usually not enough to start degradation - ubiquitination repeats until a chain of ubiquitin molecules is attached to the target protein, which serves as a signal for the proteasome - the cellular recycling center - to begin its destruction.

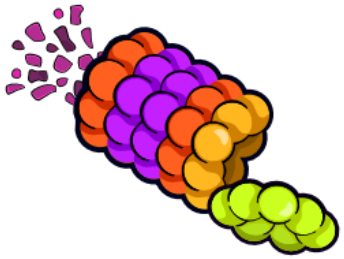
Ubiquitin depicted in the game as a circular molecule along with its actual crystallographic structure.

Most commonly, ubiquitin is attached via a peptide bond to the lysine of target proteins. However, there is also so-called non-canonical ubiquitination that can occur on other amino acids, such as tyrosine, serine or cysteine, where the binding of ubiquitin to the target protein is more unstable and its role is poorly understood.

Although the primary function of ubiquitination is to direct proteins for proteasome-mediated degradation, it is also involved in other cellular processes, including DNA repair, autophagy, signal transduction and protein transport.

7. Proteasome

The proteasome serves as a cellular recycling center, leading to the degradation of unwanted or damaged proteins. It is a large protein complex consisting of many subunits arranged in a barrel-shaped structure.



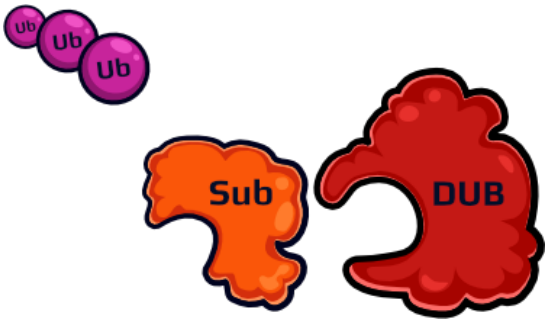
The proteasome, a cellular recycling center, recognizes ubiquitinated proteins and degrades them.

The proteasome recognizes ubiquitinated proteins and performs proteolysis, i.e., it breaks down proteins into short chains of amino acids that can be reused to synthesize new proteins. Interestingly, when the proteasome degrades a protein, ubiquitin molecules are released by deubiquitinating enzymes, so they can be reused in the ubiquitination process.

8. Deubiquitinating enzyme

Deubiquitinating enzyme (DUB) cleaves ubiquitin from proteins and reverses or modulates the ubiquitination process. There are different classes of DUBs, with varying specificity toward different ubiquitin bonds or substrates.

Although DUB acts as an antagonist in our game, proper de-ubiquitination is as important as ubiquitination for maintaining protein balance in the cell. The enzymatic activity of DUBs can be inhibited by small chemicals, which is an attractive strategy for drug discovery since DUBs have a major impact on the development of cancer and other diseases.

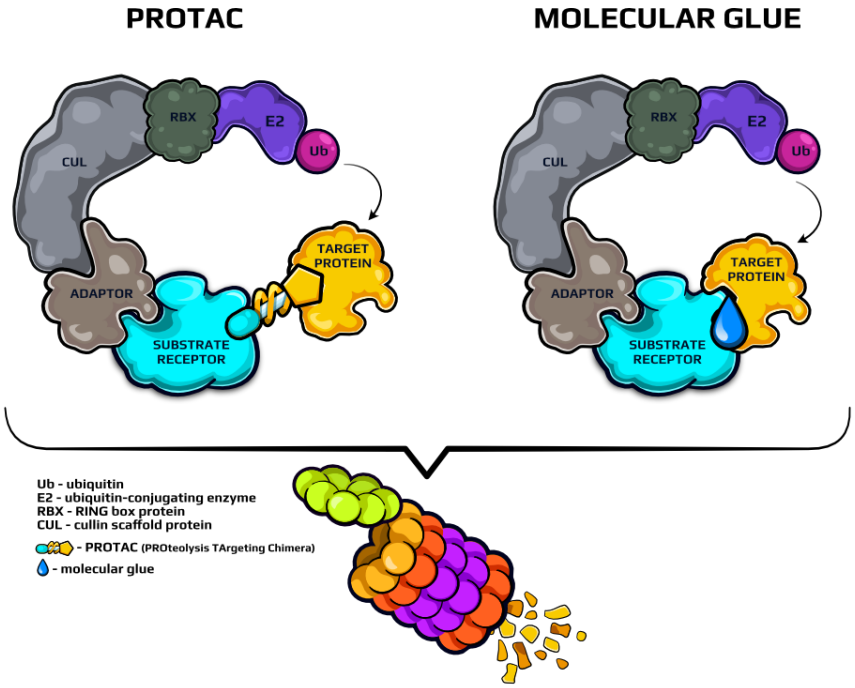


DUB enzymatically detaches ubiquitin from proteins, reversing the ubiquitination process.

9. Targeted protein degradation

Let's use our cellular degradation pathways to fight cancer!

What if we could harness our ubiquitin-proteasome system and tailor it to remove toxic disease-causing proteins? Well, this is no longer science fiction - it is how modern targeted protein degradation (TPD) therapies work. The strategy is simple; scientists design compounds that allow E3 ligases to bind proteins that they don't normally recognize. The E3 ligases then bring them to E2 enzymes, resulting in ubiquitination of the proteins and degradation by the proteasome. The two main classes of compounds using targeted protein degradation mechanisms are molecular glues and PROteolysis TArgeting Chimeras (PROTAC) compounds. Molecular glues are small molecules that enhance interactions between an E3 ligase and a selected protein, while PROTACs consist of two parts - one recognizes a specific E3 ligase, and the other recognizes the selected target protein.



Molecular glues and PROTACs are two different approaches to targeted protein degradation. Molecular glues are small molecules that enhance the interaction between the E3 ligase and the target protein. PROTACs are compounds composed of two components, in which one part recognizes the specific E3 ligase and the other part recognizes the selected target protein. Both approaches result in ubiquitination of the target protein and ultimately lead to its degradation. Although not shown in the game, molecular glues and PROTACs often use large E3 ligase complexes, particularly their subunits responsible for substrate recognition, namely the von Hippel-Lindau (VHL) or cereblon (CRBN) proteins.

Targeted protein degradation in medicine

Molecular glues, for example, lenalidomide or pomalidomide, have already achieved clinical success and are used to treat cancers such as multiple myeloma. Several PROTAC compounds are currently in advanced clinical trials against breast or prostate cancers, among others.

TPD methods make it possible to destroy disease-causing proteins, including oncogenic proteins, and hold great promise for a wide range of therapeutic applications. These technologies have such enormous potential because they make it possible to target proteins that were previously thought to be impossible to block with traditional drugs. However, this technology is not without drawbacks. For example, PROTAC compounds cannot cross the blood-brain barrier due to their size, their chemical synthesis is complex, and they can lead to unintended degradation of off-target proteins, causing side effects. Numerous studies are currently underway to optimize the performance of molecular glues and PROTAC compounds, as well as to identify new E3 ligases that can mediate targeted degradation and their protein targets. By extending the capabilities of this technique, researchers can develop new therapies for both disorders of civilization and rare diseases.