Degradation of proteins on proteasomes

Degradation of defective proteins

Proteasomes also degrade defective proteins that the cell failed to synthesize correctly (about 30% of all native proteins in the cell) and whose elimination takes place immediately during translation or shortly after it[1]. If newly synthesized defective proteins are located in the endoplasmic reticulum (ER) – mainly proteins misfolded into tertiary structures – they are subject to a process in which proteasomes cooperate with the ER and which is called **ERAD** (endoplasmic reticulum-associated degradation). During ERAD, the misfolded protein is first marked with a specific code (these are glycans), then it is transported across the ER membrane into cytosol and degraded by nearby proteasomes[2]. In the case of non-native proteins that are poorly folded and aggregated, they are taken up in cytosol using two compartments called **JUNQ** (juxta-nuclear quality control) and **IPOD** (insoluble protein deposit). Even these proteins are finally degraded in proteasomes[3]. However, proteasomes do not only degrade defective proteins, but also significantly participate in the processing of functional proteins in the sense of influencing their function. Degradation of a protein can be an important part of various signaling events in the cell, as we will see below.

Nuclear and cytoplasmatic proteasomes

Proteasomes are contained in the cytoplasm and the nucleus, where they move freely and quickly. On the other hand, the transfer of proteasomes from the cytoplasm, where there are more of them, to the nucleus (it cannot go in the other direction) is slow. During mitosis, the two separate populations of proteasomes, nuclear and cytoplasmic, can fuse and intermingle[4]. The total amount of proteasomes in a cell is in the hundreds to thousands of nanograms per milligram of all cellular proteins, although it can be significantly greater in cancer cells than in healthy cells[5]. Further details on the localization of proteasomes in the cytoplasm and nucleus can be found in the literature[6].

The ubiquitin-proteasome system

Degradation of proteins using proteasomes is a very sophisticated process that takes place within the framework of the so-called **ubiquitin-proteasome system** (UPS) or the so-called ubiquitin-proteasome pathway. An important role in this process is played by the small (76 amino acids, 8.5 kDa) protein **ubiquitin**, which was discovered already in 1975, without its function being known. However, the name already implies that this protein is abundantly found in all eukaryotic cells (it is "ubiquitous" i.e. "occurring everywhere"). Today we already know that ubiquitin serves to mark proteins in a process called ubiquitination or ubiquitinylation of a protein. The importance, diversity and complex context of protein labeling with ubiquitin today reaches the level of the most important post-translational modification of proteins, which is phosphorylation[7].

Ubiquitination processes

1. Ubiquitin is first bound (activated) in the cell when ATP is consumed by the so-called **ubiquitin-activating enzyme E1.**
2. It is subsequently transferred to the ubiquitin-transferring (or ubiquitin-conjugating) **enzyme E2**.
3. Further transfer of ubiquitin to the **protein to be degraded** (hereafter PDG; or to the forming chain of ubiquitins to PDG) is conditioned by **E3 ubiquitin ligase**, which can specifically recognize proteins to be degraded.

In other words, the E1 and E2 enzymes mainly serve only to transport ubiquitins, while the E3 enzymes handle their final attachment to PDG, recognized by these enzymes. First, the first ubiquitin is bound to the PDG, then the second, the second, the third, etc. The fact that E3 enzymes specifically recognize different PDGs also means that there are few different E1s in cells (according to [8], there are at least two E1s in human cells enzymes, namely Uba6 and Ube1) and E2 enzymes, while E3 enzymes exist in diverse numbers (hundreds) for different groups of proteins destined for degradation.

Ubiquitin-ligases

We recognize two basic genera of ubiquitin ligases, which differ in the presence of an active domain: either they contain a **HECT (homologous to E6-Associated Protein C-Terminus) domain**, or a **RING (really interesting new gene) domain**[9]_[10]. Moreover, in addition to the classic E1-E2-E3 cascade, we now also know the so-called E4 enzymes, which can participate in the extension of the polyubiquitin chain[11].

Mode of attachment of ubiquitins

A key question is also how the ubiquitins are connected to each other in the chain. Not every polyubiquitin chain is prima facie the kiss of death. The most common way of linking two ubiquitins in a polyubiquitin chain is via **lysine 48** (so-called K48 chain) or **lysine 63** (so-called K63 chain), however, there are also ubiquitin chains linked via lysine 6, 11, 27, 29 or 33, in some exceptions, these chains can even be branched[12]. Today we cannot say exactly

what the meaning of the whole variety of these signals is. K48 chains in particular are considered to be the "kiss of death", while K63 chains mainly play other roles in cell signaling. However, it has recently been shown that also proteins marked with K63 chains can be recognized by proteasomes and degraded in them[13]. There is even the possibility that K63 chains can be converted directly on the tagged protein to K48 chains[14].

Deubiquitination

In the eukaryotic cell, in addition to ubiquitination of proteins, their deubiquitination also occurs. The enzymes responsible for this process are called **deubiquitinases** and are denoted by the abbreviation DUB. We can divide them into the following five groups:

1. ubiquitin C-terminal hydrolases,
2. ubiquitin specific proteases,
3. proteases with the Machado-Joseph disease domain,
4. proteases from ovarian tumors,
5. proteases with a JAMM domain[15].

Today, 75 DUBs are known, which interact with hundreds of proteins and play an immense number of different roles in the eukaryotic cell[16]. Note that one of the JAMM domain deubiquitinases, referred to in human cells as **Poh1**, is part of the eukaryotic proteasome and plays a key role in its proper functioning.

Proteasome

Characteristics

A protein that carries a K48 polyubiquitin chain. It can be recognized by the so-called 26S proteasome and degraded in it.

The 26S proteasome is a common type of proteasome found in our cells. It consists of two basic parts[17]:

1. 20S proteasome, i.e. the **core particle**, which has the shape of a cylinder and in which the proteolysis of PDG itself takes place;
2. 19S proteasome or **regulatory particle**, which is also called PA700.

Core particle

The 20S proteasome consists of a total of four rings. The two outer ones are made up of seven α units and the two inner ones are made up of seven β subunits. The active protein-cleaving sites are in the β rings and face the inside of the 20S proteasome cylinder, namely:

- β1 subunit with caspase-like activity;
- β2 subunit with trypsin-like activity;
- β5 subunit with chymotrypsin-like activity..

In addition to the normal 20S proteasome, inducible proteasomes also exist in our cells. These have other active sites (β1i, β2i and β5i), which are called immunoproteasomes or mixed proteasomes. They play a role in the immune response of cells to foreign substances[18]. A very special type of proteasome exists in the thymus, the so-called **thymoproteasome**. They contain a β5t subunit with unusual catalytic activity. Their role is related to the positive selection of CD8+ T cells[19].

Regulatory particle

Regulatory particles bind to the outer, i.e. α ring of the 20S proteasome. In addition to the 19S proteasome, these can also be other complexes, such as PA28 or PA200, or even proteins that reversibly attach to the 20S proteasome in substoichiometric amounts[20]. Although the organization of proteasomes varies dynamically, it has been shown that the 26S proteasome remains intact during protein degradation[21].

The 26S proteasome regulatory particle (PA700) contains two basic, interconnected regions: a **base** and a **lid**. In the base we can find six different AAA+ ATPases and another four subunits. Its main mission is to regulate entry into the interior of the 20S proteasome[22]. The lid contains nine non-ATPase subunits[23] and its primary function is the deubiquitination of ubiquitinated proteins by the JAMM domain DUB Poh1 before their entry into the interior of 26S proteasomes[24].

Degradation of non-ubiquitinated proteins

A typical protein, degraded in a eukaryotic cell by the proteasome, must be ubiquitinated. However, according to recent findings, about 20% of all proteins cleaved by proteasomes in eukaryotic cells may not have ubiquitin labeling. Such proteins contain disordered sites in their structures that serve as a non-specific signal for degradation in proteasomes without the need for ubiquitination of the given protein[25].

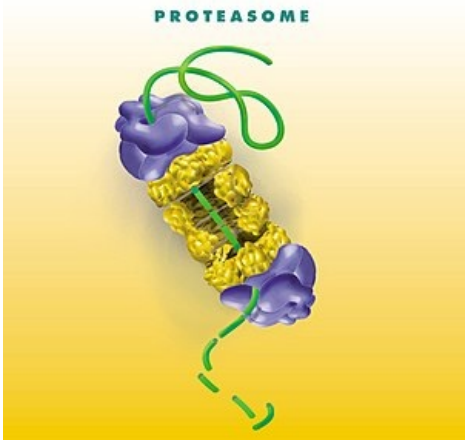
Degradation of ubiquitinated proteins

We will focus on the mechanism of degradation of ubiquitinated PDGs in 26S proteasomes.

Some subunits from the base (ubiquitin receptors) and also some proteins that only transiently associate with 26S proteasomes play a key role in the recognition of the ubiquitinated protein[26]. If the ubiquitinated protein is already bound to the 26S proteasome, its polyubiquitin chain can be variously cleaved by the proteasome and resynthesized by deubiquitinases and ubiquitin ligases[27]. It has also been shown that the reduction in the intensity of degradation of ubiquitins themselves in the proteasome is related to the activity of a specific DUB, called Ubp6, which is not a constant subunit of the 26S proteasome[28].

Steps of the degradation

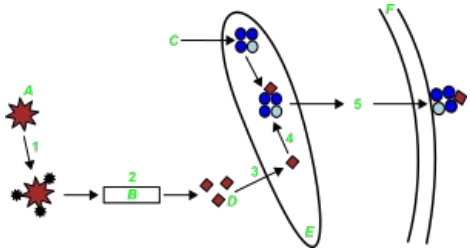
- Before PDG degradation itself, the polyubiquitin chain is usually cleaved en bloc (as a whole, at once) by **Poh1** and further removed by other DUBs[29].
- The unraveling of the protein into the primary structure and its movement into the opening of the proteasome is then connected with the hydrolysis of ATP by AAA+ ATPases[30].
- The unfolded protein can be "stored" in the α rings if the β rings are still occupied by the degradation of the previous PDG[31]. Proteins can enter the 20S proteasome from both sides[32]. Degradation takes place until the resulting oligopeptides are small enough to spontaneously diffuse out[33].
- As soon as the oligopeptides get out of the 26S proteasomes, they are further cleaved in the cell by other peptidases to amino acids that can be used for further proteosynthesis[34], or are used within the immune system as antigens[35].



Proteasome

Regulation of protein activity

Some proteins are not completely degraded by 26S proteasomes, but are actually activated. This happens by degrading other proteins that are bound to them and inhibit them. A typical example is the activation of the so-called nuclear factor-κB (NF-κB), which normally occurs in the cytoplasm in a complex with its inhibitor I-κB. Once the I-κB is ubiquitinated and degraded, NF-κB translocates to the nucleus and triggers the transcription of the relevant genes[36]. The function of 26S proteasomes is not only connected with the regulation of the amount of a given protein in the cell, but also with the regulation of the activity of various proteins. It follows that the UPS plays a key role in many therapeutically important processes, such as inflammatory diseases, neurodegenerative processes, muscular dystrophies, viral infections or carcinogenesis[37]-[38].



The role of the proteasome in antigen presentation:
A – Protein
B – Proteasome
C – MHC I protein synthesis
D – Peptides to be presented
E – ER
F – Plasmatic membrane
1. Ubiquitination
2. Degradation of protein into peptides by the proteasome
3. Peptide transport into the ER lumen by ABC transporters
4. Binding of peptides in the notch of the MHC I complex
5. Antigen presentation on the plasma membrane

Proteasome inhibitors

Proteasome inhibition leads to apoptosis of some tumor cells. Although the exact mechanism of their action on cells is not yet known, they are used as antitumor drugs. The most prominent representative is bortezomib, which is used to treat multiple myeloma.

Links

Related articles

- Proteasome inhibitors / history
- Proteasome inhibitors/study
- Proteasome inhibitors / 2. generation
- Proteasome inhibitors / Antabuse (Disulfiram)
- Lysosomes
- Cancerogenesis

Taken from

- CVEK, Boris. Od ubikvitinu k antabusu. *Britské listy : deník o všem, o čem se v České republice příliš nemluví* [online]. 2011, vol. -, p. -, Available from <https://blisty.cz//legacy.blisty.cz/art/56680.html>. ISSN 1213-1792.

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