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Paradigms of protein degradation by the proteasome

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The proteasome is the main proteolytic machine in the cytosol and nucleus of eukaryotic cells where it degrades hundreds of regulatory proteins, removes damaged proteins, and produces peptides that are presented by MHC complexes. New structures of the proteasome particle show how its subunits are arranged and provide insights into how the proteasome is regulated. Proteins are targeted to the proteasome by tags composed of several ubiquitin moieties. The structure of the tags tunes the order in which proteins are degraded. The proteasome itself edits the ubiquitin tags and drugs that interfere in this process can enhance the clearance of toxic proteins from cells. Finally, the proteasome initiates degradation at unstructured regions within its substrates and this step contributes to substrate selection.

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Introduction

Cellular protein concentrations are controlled through their rates of synthesis and degradation. In the cytosol and nucleus of eukaryotic cells, most of this degradation is by the ubiquitin proteasome system (UPS). At the center of the UPS is a single proteolytic machine, the proteasome, which controls the concentrations of hundreds of regulatory proteins and clears misfolded and damaged proteins from the cell. Thus, the proteasome has to be able to degrade any protein but do so while avoiding the accidental destruction of the rest of the cellular proteome. Here we review recent advances in our understanding of how the proteasome selects its substrates. Just as protein synthesis is regulated at many different levels, it is becoming increasingly clear how protein degradation is also.

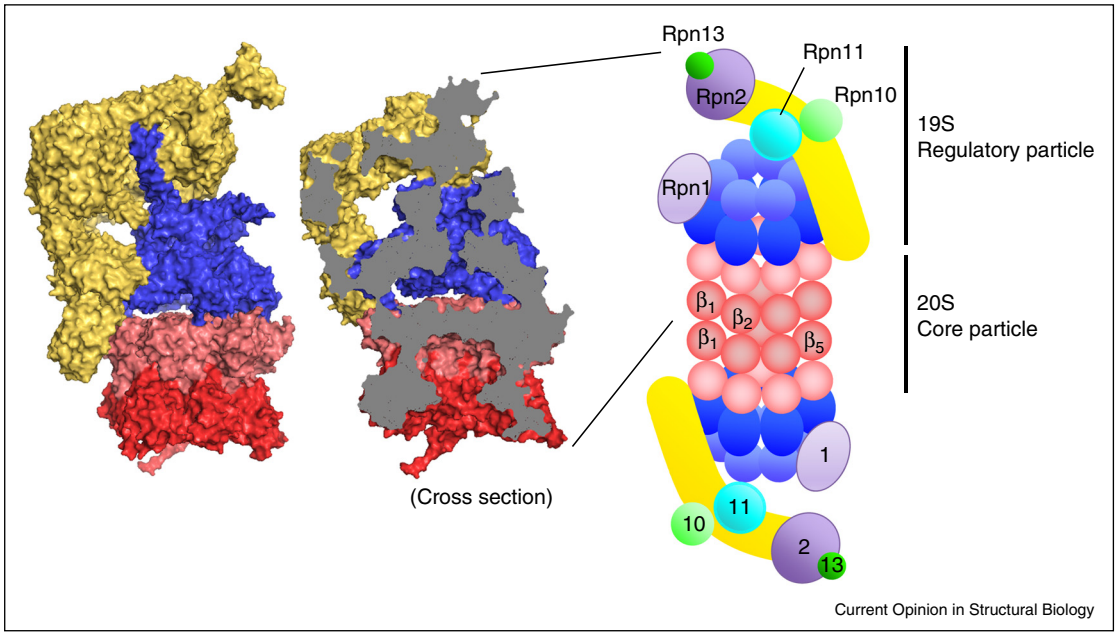
The basic principle of proteasome substrate selection is well understood [1,2]. The proteasome is a large particle of ~33 different subunits that add up to a molecular weight of approximately 2.5 MDa. It combines three different proteolytic sites with broad and complementary sequence preferences to allow it to degrade many different amino acid sequences. The proteasome particle controls the activity of these sites by encapsulating them inside its structure and controlling access to them. Most proteins are targeted to the proteasome by the covalent attachment of ubiquitin molecules. The proteasome recognizes the ubiquitin signal and initiates degradation at an unstructured region in the protein. The substrate is then unfolded and translocated to the proteolytic sites in an ATP-dependent reaction. However, many questions remain. For example, the proteasome is able to extract individual subunits from complexes without degrading their binding partners, the proteasome degrades ubiquitinated proteins in a specific order and ubiquitin signals target proteins to processes that do not involve degradation. We do not know how the proteasome makes these distinctions. At the same time, some proteins that lack ubiquitin signals are degraded by the proteasome. Over the last few years, new proteasome structures and biochemical investigations have brought new insights into these questions.

Proteasome

The proteasome particle is functionally and structurally divided into two parts. Its core is formed by a cylindrical 20S particle composed of four heptameric rings that are stacked onto top of each other. The inner two rings each consist of seven related β-subunits that are arranged to form a large internal cavity and three of the subunits in each ring contain a proteolytic site that faces the internal cavity. A ring of seven related α-subunits on each side flanks the β-rings and substrates have to enter the proteolytic cavity formed by the β-rings through a pore at the top of the α-ring. The pore is too narrow to allow folded proteins to pass through it. In free core particle, access to the pores is further hindered by the N-termini of the α-subunits so that even unfolded peptides are degraded only poorly.

The core particle is activated by regulatory particles or caps that bind to the ends of the core particle and induce conformational changes that open the pores. Four different caps are known and the best understood of them is the19S regulatory particle. It consists of 19 subunits that add up to a molecular weight of ~900 kDa. The complex of one or two of these caps with the 20S core particle is called the 26S proteasome and this seems to be the most common form of the proteasome in cells. The subunits of the 19S cap recognize substrates, unfold and translocate

Figure 1



Structure of the 26S proteasome. Molecular surface of the 19S activator particle bound to the 20S core particle (PDB 4C0V) (left). The 20S core particle is composed of two central β rings (dark red) and one α ring (light red) at each end. The 19S regulatory particle, which contains AAA ATPase subunits (blue) and non-ATPase subunits (yellow), caps each end of the 20S. Cross section reveals the degradation channel that connects the proteolytic chamber in the 20S core particle to the entrance into the 19S activator (middle). Structures are produced by PyMOL. Schematic drawing of the 26S proteasome indicates the approximate locations of the enzymatic activities and binding platforms on the 19S activator cap (right). α (light red) and β (dark red) subunits of the 20S particle, ATPase domain (dark blue) and OB domain (light blue) of ATPase subunits, backbone of lid subparticle (yellow), docking subunits Rpn1 (light purple) and Rpn2 (dark purple), ubiquitin receptors Rpn10 (light green) and Rpn13 (dark green), and DUB metallo-protease subunit Rpn11 (sky blue).

them into the core particle for degradation into short peptides.

Structure of the 26S proteasome

The structure of the 26S proteasome proved difficult to determine, perhaps because a number of accessory factors associate with the particle non-stoichiometrically or because the structure undergoes conformational changes. In a major breakthrough, a series of studies published over the last two years describe the structure of the 19S cap bound to the core particle at high resolution by combining cryo-electronmicroscopy, crystallography, biochemical data and computer modeling [3••,4••,5••,6••,7••,8••,9••,10••] (Figure 1).

The heart of the 19S cap is a ring of six ATPase subunits (Rpt1–Rpt6), which make up the motor that feeds substrates to the proteolytic sites. The subunits form a long channel at their center that runs through approximately two-thirds of the 19S particle and ends in a ring of the AAA+ domains at the C-terminal end of the ATPase subunits. The very C-termini of the AAA+ domains dock into the 20S core particle and trigger pore opening. Two large subunits that serve as interaction platforms bind to the ATPase ring, Rpn1 to the outside of the ring, and Rpn2 to the top of the ring. Rpn1 provides the binding sites for a series of non-stoichiometric proteasome subunits called UbL-UBA proteins, which serve as additional ubiquitin receptors and we will discuss these briefly later, and Rpn2 organizes the two ubiquitin receptors Rpn10 and Rpn13 subunit near the outer end of the 19S cap. No single one of these receptors is essential in yeast [11••] so that it seems that the different receptors work together to form a versatile binding platform to capture proteasome substrates (Figure 3). The cap also contains a pair of JAMM or MPN domain metallo-protease subunits called Rpn11 and Rpn8. Only Rpn11 is enzymatically active and it cleaves entire ubiquitin chains off the substrates as these are degraded. Rpn11 is located near the entrance of the substrate channel formed by the ATPase subunits so that it is well placed to interact with substrate protein feeding into the proteasome. Thus, the activities required for protein degradation are ordered sequentially along the long axis of the proteasome particle [2] (Figure 1).

The remainder of the cap is formed by seven scaffolding subunits that form a clamp that binds to the side of the cap reaching all the way from the end of the proteasome particle, where it interacts with Rpn2 and the ubiquitin receptor Rpn10, via the ATPase subunits, down to the α-ring of the