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Review

# Regulation of proteasome activity in health and disease ☆

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ABSTRACT

The ubiquitin–proteasome system (UPS) is the primary selective degradation system in the nuclei and cytoplasm of eukaryotic cells, required for the turnover of myriad soluble proteins. The hundreds of factors that comprise the UPS include an enzymatic cascade that tags proteins for degradation *via* the covalent attachment of a poly-ubiquitin chain, and a large multimeric enzyme that degrades ubiquitinated proteins, the proteasome. Protein degradation by the UPS regulates many pathways and is a crucial component of the cellular proteostasis network. Dysfunction of the ubiquitination machinery or the proteolytic activity of the proteasome is associated with numerous human diseases. In this review we discuss the contributions of the proteasome to human pathology, describe mechanisms that regulate the proteolytic capacity of the proteasome, and discuss strategies to modulate proteasome function as a therapeutic approach to ameliorate diseases associated with altered UPS function. This article is part of a Special Issue entitled: Ubiquitin–Proteasome System.



## 1. Introduction

The cellular protein pool is in constant flux. Its composition is defined by the proteostasis network, comprised of ribosomes, chaperones and two proteolytic systems, the lysosome and UPS [1]. These adaptable systems sustain protein homeostasis under a large variety of different conditions such as during oxidative stress, cellular differentiation, varying nutrient supply, exposure to elevated or reduced temperature, and xenobiotic stress. The activities of the different components of the proteostasis network are functionally linked and compensatory strategies are in place to avoid proteostasis collapse if the activity of one or more of the network components declines. Failure or malfunction of the proteostasis network is often associated with human disease [1]. Modulating proteostasis has therefore emerged as a promising new avenue for the development of treatments for diverse diseases such as cancer, neurodegeneration, autoimmunity, cardiomyopathy, inherited diseases caused by partial loss of function mutations, such as cystic fibrosis, and inherited diseases associated with protein misfolding and toxic gain of function.

The UPS is the primary degradation system that mediates the degradation of short-lived regulatory proteins and the removal of damaged soluble proteins. The recognition signal for proteasomal degradation is a ubiquitin chain covalently attached to lysine residues in substrate proteins. Formation of an isopeptide bond between the ε-amino group of substrate lysines and the carboxyl group of the C-terminal glycine of ubiquitin is an ATP-dependent process and is achieved *via* an enzymatic cascade involving three distinct classes of enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3) [2]. The same reaction links additional ubiquitin molecules to the primary ubiquitin *via* internal ubiquitin lysines, thus creating a ubiquitin chain. A chain of at least four ubiquitins linked *via* lysine 48 is the classical recognition motif for proteasomal degradation [3], though chains of other linkage types are now recognized as physiological targeting motifs, and for some substrates multiple monoubiquitin modifications are sufficient for targeting to the proteasome [4,5]. The proteasome holocomplex or 26S proteasome consists of two entities: a central cylindrical structure with peptide hydrolysis activity (core particle (CP) or 20S proteasome) and a regulatory particle (RP, also known as the regulatory cap, 19S particle, or PA700), required for substrate recognition, removal of the ubiquitin chain and ATP-dependent unfolding.

Medical interest in modulating proteasome function for therapeutic purposes has significantly increased during the past decade since the first proteasome inhibitor, Velcade/Bortezomib, has been approved by the Food and Drug Association (FDA) in 2003 for the treatment of refractory multiple myeloma [6]. Studies that led to the FDA approval demonstrated that the proteasome can be transiently and safely inhibited in humans with anti-tumor activity especially against hematopoietic malignancies [6]. The efficacy of proteasome inhibition for the treatment of cancer is based on its role in regulating cell proliferation and on the exquisite reliance of cancer cells on proteasome function [7]. However, many diseases, especially neurodegenerative diseases are characterized by the accumulation of toxic misfolded proteins and eventual collapse of the proteostasis network. Thus, both proteasome inhibition and activation represent potential avenues for future drug

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development. During the last two decades many mechanisms that regulate the UPS have been unraveled. Mechanisms that specifically modulate the proteolytic activity of the proteasome are discussed in this review.

## 2. Major alternate forms of the proteasome

The central assembly of the proteasome is a cylindrical structure that houses the proteolytic active sites. The cylinder is formed by two different types of subunits, α and β, which are arranged in four stacked heptameric rings enclosing a central cavity. In eukaryotes, seven distinct α subunits are located in the two outer rings of the barrel, and seven distinct β subunits form the two inner rings [8]. Three of the β subunits contain active sites with different peptide cleavage specificities. β1 has a caspase-like specificity, cleaving after acidic residues; β2 is trypsin-like, cleaving after basic residues; and β5 is chymotrypsin-like in cleaving after large hydrophobic residues. Their active sites face the interior of the cylinder. Access to the central cavity is regulated by an adjustable gate that is formed by the N-terminal protrusions of the α subunits [9,10]. The default status of the CP gate is closed and for proteasomal degradation and substrate access the N-termini need to be displaced from their axial position to reveal a continuous channel leading into the catalytic cavity. Modulation of the gate is a prerequisite for substrate entry into the proteolytic chamber and is mediated by proteasome activators. Several proteasome activators have been described, including the regulatory particle (RP/19S/PA700), activators of the PA28 protein family and Blm10/PA200 activators [11]. All activators share common binding sites on the two outer surfaces of the CP/20S (Fig. 1). The binding pockets for activators are formed by interfaces of adjacent α subunits. The molecular mechanism of displacing the N-termini of the α subunit, which results in gate opening, appears to be different for the different activator families, and will be discussed below.

It should be noted that in the absence of activators the free CP shows low but detectable activity towards small proteins with intrinsically unstructured regions in the absence of ubiquitination and ATP [12]. The significance of this activity is not understood but it may reflect the occurrence of spontaneous, short-lived opening of the gate at some frequency [13].

### 2.1. RP–CP: the proteasome holoenzyme

The dominant proteasome activator is the RP. It promotes ATP- and ubiquitin-dependent substrate turnover. The RP can bind to either one or two ends of the CP to form the proteasome holocomplex or 26S proteasome. The RP is composed of 19 integral subunits, which form two biochemically separable sub-complexes, lid and base [14]. The base subcomplex is situated proximal to the CP gate region. It contains six homologous ATPases (Rpt1–Rpt6), which form a hexameric ring. They belong to the AAA family of ATPases. The ring is not planar but has a spiral staircase-like topology, with Rpt3 at the highest and Rpt2 at the lowest position [15–17]. In addition to the ATPases, the base contains the two largest RP subunits, Rpn1 and Rpn2, and the two ubiquitin-receptors Rpn10 and Rpn13 [18]. The lid consists of nine non-ATPase subunits. One Rpn subunit, Rpn6, appears to contact both the base as well as the α ring and might be a crucial regulator of lid–base assembly [15,19]. One of the non-ATPases, Rpn11, has deubiquitinating activity and is located in close proximity to the substrate entry pore formed by the ATPase ring [15]. Its deubiquitinating activity is required to promote the degradation of ubiquitinated proteasome substrates [16,20–22].

Cryo-EM studies have defined two conformational states of the RP, ATP-h and ATP-γS. The ATP-h structure, the first to be defined [15,17] is now seen as likely to represent a basal state of the enzyme. This structure is defined by the presence of ATP in the buffer and the absence of

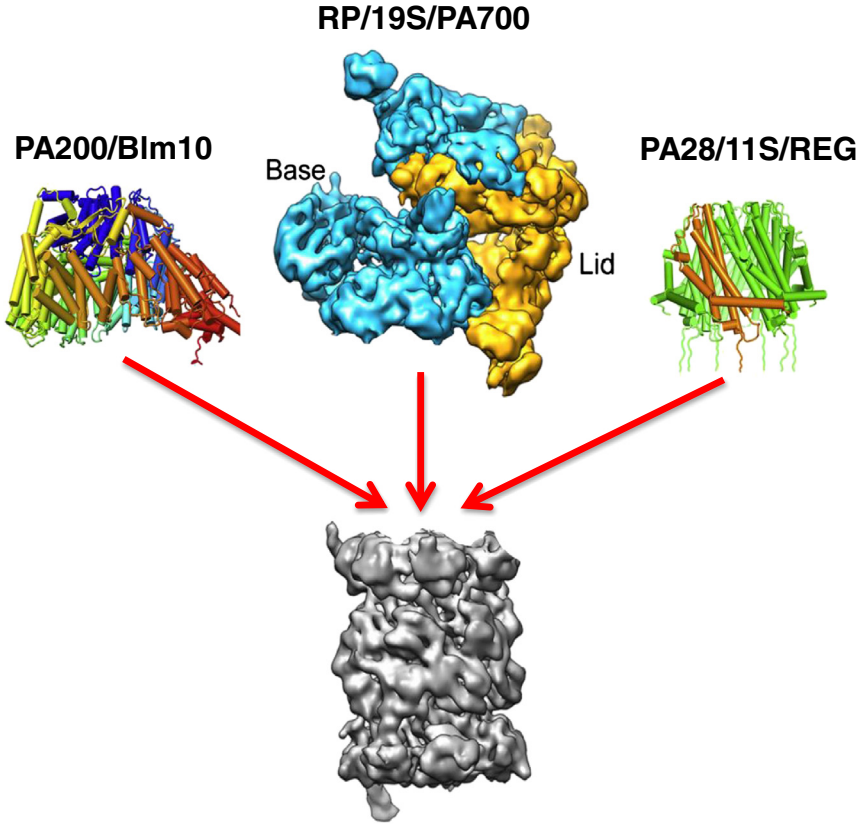


**Fig. 1.** Modular structure of proteasome complexes. The proteasome core particle can be capped with three different activator complexes: the hetero-oligomeric RP/19S/PA700 particle, the monomeric Blm10/PA200 proteins and the heptameric PA28/11S/REG rings (adapted from [11] and [15] with permission). Activators from all three families occupy the same binding site at the apical surface of the CP and open the CP gate.

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