Trends in Biochemical Sciences

CellPress

Special Issue: 40 Years of TiBS

Review

Gates, Channels, and Switches: Elements of the Proteasome Machine

Daniel Finley,[1,*] Xiang Chen,[2] and Kylie J. Walters[2,*]

The proteasome has emerged as an intricate machine that has dynamic mechanisms to regulate the timing of its activity, its selection of substrates, and its processivity. The 19-subunit regulatory particle (RP) recognizes ubiquitinated proteins, removes ubiquitin, and injects the target protein into the proteolytic chamber of the core particle (CP) via a narrow channel. Translocation into the CP requires substrate unfolding, which is achieved through mechanical force applied by a hexameric ATPase ring of the RP. Recent cryoelectron microscopy (cryoEM) studies have defined distinct conformational states of the RP, providing illustrative snapshots of what appear to be progressive steps of substrate engagement. Here, we bring together this new information with molecular analyses to describe the principles of proteasome activity and regulation.

The Proteasome

The proteasome is the most complex protease known and, with hundreds of substrates, the scope of its regulatory functions is likewise unparalleled. Its principal activity is to degrade proteins conjugated to ubiquitin. The proteolytic sites of the proteasome are sequestered within an internal chamber [1], which is essentially a topological compartment defined by the inner surfaces of the 28 subunits that form the proteasome CP (also known as the 20S particle) (Figure 1A,B). Cellular proteins have highly restricted access to this chamber and the proteolytic sites within it, thus minimizing nonspecific proteolysis.

In the proteasome holoenzyme, the CP is complexed with the 19-subunit RP (also known as the 19S particle and PA700) (Figure 2A). The RP contains receptors for ubiquitin thus initiates substrate recognition. After conjugated ubiquitin is tethered to the RP, the attached substrate is translocated from the RP to the CP. Translocation requires ATP hydrolysis, which is effected by a heterohexameric ring of ATPases that are subunits of the RP. Here, we describe current understanding of the key steps in protein breakdown by the proteasome. Although it degrades hundreds of proteins, the proteasome is highly selective, and we discuss the structural elements that restrict its activity to proper substrates. Finally, we review the results of recent cryoEM studies that have highlighted the plasticity of the proteasome and revealed broad conformational switches that serve to orchestrate its diverse catalytic activities, including ATP hydrolysis, deubiquitination, and substrate proteolysis.

The Catalytic Core Particle

In the CP, four stacked heteroheptameric rings of subunits are assembled into an $\alpha_7\beta_7\beta_7\alpha_7$ architecture (Figure 1A,B). Thus, the outer rings are formed by the α subunits and the inner rings by β subunits. Three of the seven β subunits are proteolytically active, cutting after hydrophobic

Trends

Docking of a substrate at the proteasome RP complex is mediated by ubiquitin recognition, but, to be degraded, the substrate must be translocated through a channel leading from the RP to the proteolytic CP complex.

Given that the channel from the RP to the CP is narrow, translocation generally requires unfolding of the substrate. Hydrolysis of ATP supplies the mechanical force required for substrate unfolding and translocation.

Protein loops that line the channel within the RP interact with substrate and move axially to direct vectorial motion of the substrate towards the CP.

Ubiquitin promotes degradation, but, if not removed, can impede translocation because it resists unfolding.

Ubiquitin is removed from the substrate either before substrate entry into the translocation channel or contemporaneously with this event, depending on the deubiquitinating enzyme. Rapid deubiquitination can preempt substrate degradation.

Recent cryoEM studies indicate that the proteasome adopts distinct conformational states, which appear to be distinguishable as substrate-receiving or substrate-engaged states.

[1]Department of Cell Biology, Harvard Medical School, 240 Longwood Ave, Boston, MA 02115, USA
[2]Protein Processing Section, Structural Biophysics Laboratory,

Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA

*Correspondence:
daniel_finley@hms.harvard.edu (D. Finley) and kylie.walters@nih.gov (K.J. Walters).

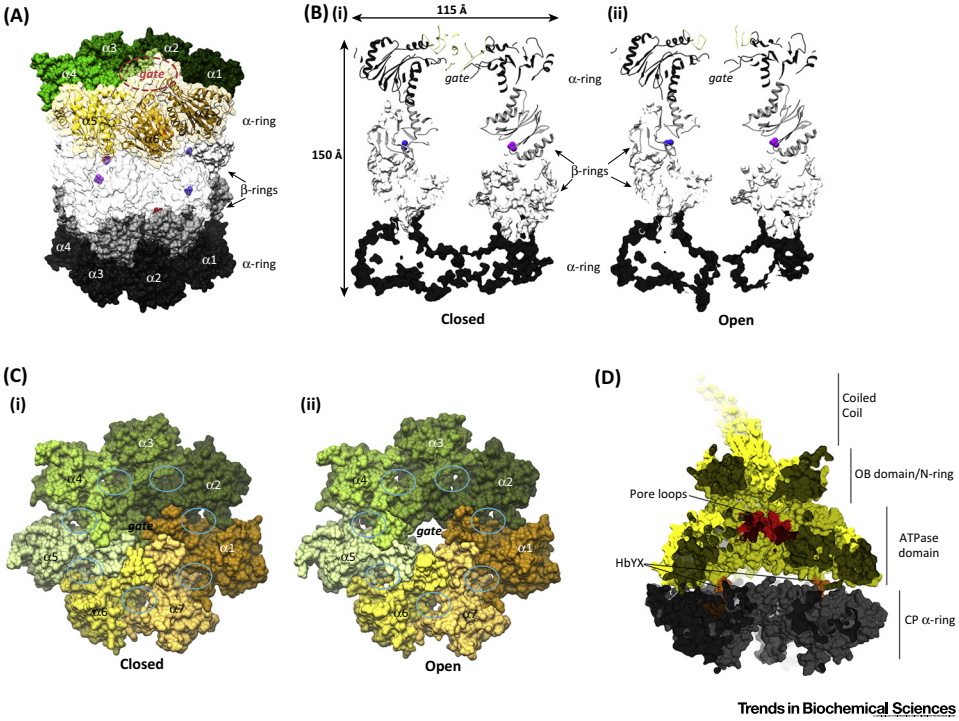

Figure 1. Substrate Entry into the Core Particle (CP) Is Gated by N-Terminal Tails of the ∝ Subunits. (A) A semitransparent surface representation of the proteasome CP, generated using Protein Data Bank (PDB) 1RYP [1]. For the top ∝-ring, ribbon diagrams are included for ∝5–∝7 and the gating residues circled. The top and bottom β-rings are distinguished by white and gray coloring respectively, and the N-terminal β1, β2, and β5 catalytic threonines rendered in blue, red, and purple, respectively. The surfaces of ∝5–∝7 and β5–β7 of the top rings are transparent for visualization of the catalytic sites. (B) A cross-section of the CP in its closed (i) and open (ii) state to illustrate the hollow interior and altered configuration of the N-terminal gating residues. The top ∝- and β-rings are rendered as ribbon diagrams, whereas the bottom two rings are displayed as surfaces. The β1 and β5 catalytic sites are represented in blue and purple, respectively. Dimensions are provided for scale. (C) Top view of the ∝-rings for closed (i) and open (ii) configurations of the CP. The intersubunit clefts where C termini of many proteasome regulators dock, including the regulatory particle (RP), are circled and the gate labeled. PDB 1RYP [1] was used for the CP closed state represented in (A–C) whereas PDB 1FNT [2] was used for the open state in (B,C). The latter structure was solved in complex with the PA26 regulatory particle. (D) Surface diagram cut-away view of the CP ∝-ring (gray) with the RP ATPase ring (yellow) highlighting the pore loops (red), hydrophobic-tyrosine-X (HbYX) motifs (orange), and contiguous substrate entry channel. PDB 4CR4 [53] and 1FNT [2] were used to generate this image.

(β5), basic (β2), or acidic (β1) residues (Figure 1A,B). The combination of limited specificity and multiple active sites ensures rapid degradation once substrates enter the chamber of the CP. In the crystal structure of the free CP (i.e., with the RP absent), the hydrolytic chamber is essentially closed to its environment [1]. However, a pathway for substrate entry can be formed axially at the faces of this barrel-like structure [2,3]. This translocation channel is gated, in that it exists in open and closed states that can be interconverted. In the free CP, the gate is closed (Figure 1A), because this site is occupied by coalescence of the short, highly conserved N-terminal tails from the seven ∝ subunits [3] (Figure 1A,Bi,Ci). When the RP binds the CP, it is positioned over the CP channel and opens the gate, relieving autoinhibition of the CP (Figure 1Bii,Cii,D).

The translocation channel is narrow [2,3], ensuring that, even in its open state, properly folded cellular proteins are excluded from the hydrolytic chamber of the CP. To traverse the channel, substrates must typically be unfolded by the ATPase ring of the RP (the Rpt ring), as is the case for ATP-dependent proteases in general [4–7]. Globular domains of the substrate are denatured by being forced through the axial channel of the Rpt ring; unfolding is driven mechanically by