Spotlight

Insights into Proteasome Conformation Dynamics and Intersubunit Communication

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A recently published paper applies cryo-electron microscopy (EM) studies and biochemical/genetic approaches for the elucidation of the mechanisms linking nucleotide binding by ATPases, proteasome conformation dynamics, and gate opening of the 20S core particle. These insights potentially represent a milestone in our understanding of the structural dynamics of the 26S proteasome.

The dynamics through which the 19S regulatory particle couples its substrate-processing activity with the opening of the 20S core particle gate during the 26S proteasome catalytic cycle is the focus of investigation by experts in the field of proteasome structural biology.

The ‘rigid’ structure of the 20S (comprising four stacked rings, two outer α and two inner β, each of seven repeated subunits, named α\textsubscript{1–7} and β\textsubscript{1–7}), which houses the catalytic activity, renders this assembly suitable for crystallographic resolution. Thus, it is well known that the narrow central pore through which substrates are pulled for catalysis is gated by the N-terminal tails of the seven α-subunits: furthermore, this gate-keeping mechanism displays a significant degree of conservation across evolution.

However, this extensive knowledge is in contrast to the persistent difficulty in determining the structure of the 19S (and, thus, of the 26S proteasome), which can be subdivided into two modules, the lid (involved with substrate recognition) and the base, mainly constituted by ATPases (Rpt1–6; forming a pore loop laying above the α-ring of the 20S and involved in substrate unfolding and translocation). In these instances, highly dynamic properties that render crystallographic analyses problematic. Therefore, the mechanism by which ATPases carry out the ATP-dependent unfolding and translocation of polyubiquitinated substrates into the 20S, and the associated remodeling of the α-ring configuration, can only be unraveled by using alternative methodological approaches to traditional X-ray crystallography. Thus, cryo-EM coupled with integrative modeling has emerged as a fascinating and potent tool to investigate these unresolved structures and mechanisms.

In their recent paper \cite{1}, Eisele et al. applied cryo-EM and biochemical/genetic approaches to the yeast 26S proteasome to cast light on the mechanisms linking nucleotide binding by ATPases, proteasome conformation dynamics, and gate opening of the 20S core particle. Their findings potentially represent a milestone in our understanding of the structural dynamics of the 26S proteasome.

The study is the last in a series that has provided detailed structural insights into the different configurations of the 19S in the presence of ATP (and ATP analogs or substrates) throughout the catalytic cycle of the 26S. The existence of these configurations appears to be conserved among eukaryotic proteasomes, and the 19S modules clearly arrange into spatially and chronologically defined configurations that are energetically favored to accomplish substrate unfolding and translocation, and 20S gate opening.

In Archaea, the access of substrates to the 20S is regulated by the PAN-ATPase complex (the homolog of the 19S eukaryotic regulatory particle), which contains a conserved C-terminal hydrophobic-tyrosine-X (HbYX) motif that inserts into the α-pocket, thus triggering gate opening \cite{2,3}. However, the engagement of the subunits of yeast 19S that have these motifs (the ATPase Rpt2, Rpt3, and Rpt5 subunits) is not sufficient to stimulate the gate opening of the yeast 20S \cite{4}.

The study by Eisele et al. provides a convincing allosteric model in which the docking of the C-terminal tails of Rpt1 and Rpt6 into the α4–α5 and α2–α3 pockets, respectively, of the 20S induces the structural rearrangement necessary for gate opening (Figure 1). It has long been debated whether the recruitment of ATPases is stochastic or spatially and temporally ordered. This study provides a further structural clue that, in the presence of ATP, the recruitment of ATPases is hierarchical. Moreover, the Rpt2 subunit likely intervenes in the early phase of the cycle and its position in the ring appears to be well suited to coordinate the dynamics of Rpt1–Rpt6 insertion into the 20S.

Furthermore, mutations in ATPase subunits, such as Rpt4 and Rpt5, which render them unable to hydrolyze ATP, were found to be lethal in yeast cells, suggesting that these subunits have a critical role in the structural dynamics of the proteasome in steps of the catalytic cycle that occur in the presence of natural substrates in vivo.

Another important result of the work by Eisele and collaborators is the identification of two previously unreported conformational states of the 26S proteasome...
(named s5 and s6) in addition to the four already identified (s1–s4) [4–6]. The authors proposed an ATP-driven functional model that allows the proteasome to shift from the grounded s1 state in the absence of substrate to a primed state (s2 or s5) after the binding of substrate, and, finally, to the activated states (s3, s4, and s6), where substrate is translocated into the catalytic chamber to be degraded.

Recently, in a closely related study, the cryo-EM structure of the activated human proteasome also revealed the existence of six alternative conformational states in the human proteasome similar to that observed in yeast, the architectural transition of which is mediated by nucleotide binding to the AAA-ATPase ring [7]. In addition, some of the conformational states have been further documented in living hippocampal neurons, enabling researchers to discern the functional status of the proteasome particles in vivo [8].

The study by Eisele et al. also stimulates the interest on the structural phenomena related to the conformational transition occurring, after the binding of the first 19S, on the opposite end of the 20S, where a free α-ring surface is available for the binding to a second 19S particle, which would yield the doubly-capped proteasome. Although this aspect of the formation of the two capped assemblies is not characterized yet, the article introduces this point as a future main field of investigation.

The identification of different structurally defined proteasome states appears to be pivotal to pinpoint synthetic or natural allosteric modulators of proteasome activity, which could influence the equilibrium between different conformations promoting or preventing the active states independently in different pathophysiological conditions. Moreover, since the percentage of active proteasome is regulated by different conditions (stress, aging, metabolic conditions, or cell cycle), the conformational variability and relative abundance of proteasome particles in a specific state should also reflect the functional state of the cell.

References