Large, multisubunit machines carry out many fundamental biochemical processes in eukaryotic cells. Examples include the spliceosomes that remove introns from mRNA precursors, ribosomes that synthesize proteins, and proteasomes that degrade intracellular proteins. Most such machines use ordered conformational changes to coordinate the different reaction steps catalyzed. Structural information from these dynamic megadalton-sized particles has begun to pour in following recent technical advances in cryoelectron microscopy (cryo-EM). For the proteasome, three recent papers, two in PNAS, have provided atomic-resolution views of this ∼2.5-MDa complex by cryo-EM methods (1–3).

A particularly valuable feature of cryo-EM is that it allows for the reconstruction of distinct conformational states from the same sample or from proteins purified under different conditions (4, 5); these conformations can often be tentatively assigned to particular functional states. The new study on human proteasome structure by Chen et al. (3) proposes four alternative conformational states based on classification of particle images using maximum likelihood methods, yielding new insights in proteasomal mechanics.

In the ubiquitin-proteasome system, proteins that must be degraded are first tagged with polymers of ubiquitin through an enzymatic cascade; the ubiquitylated proteins are then targeted to the proteasome (6, 7). The proteasome holoenzyme is an ATP-dependent protease complex composed of ∼33 different polypeptides and assembled from several subcomplexes (8). The barrel-shaped 20S proteasome core particle (CP) bears the protease active sites in a central chamber accessible only through narrow gated channels at the ends of the cylinder. On one or both ends of the CP sits a 19-subunit regulatory particle (RP); the RP is responsible for opening the central gate in the outer (α-subunit) ring of the CP. The RP itself comprises a “base” that includes a heterohexameric ring of AAA ATPases (ATPases associated with diverse cellular activities) and a “lid” with a metalloenzyme subunit, Rpn11 (RP non-ATPase subunit 11), that removes ubiquitin chains en bloc from substrate proteins (9). The new structural data show that the interfaces between these different subcomplexes are under dynamic regulation, leading to the ordered passage and unfolding of substrates in the RP, followed by their translocation into the CP interior, where they are cleaved into short peptides.

Before the current crop of atomic or pseudoatomic structures, subnanometer cryo-EM reconstructions, mostly of yeast proteasomes, yielded hints about some of the basic features of proteasome mechanics (5, 9). These studies complemented a large body of biochemical and genetic investigations of the complex (7). Substrates are first bound by intrinsic subunits of the RP or by so-called shuttle factors, which are proteins with ubiquitin-binding domains that cycle on and off the proteasome, releasing their substrates on the proteasome surface. Binding does not lead to degradation of the substrate protein unless it contains a weakly folded or disordered element. This unfolded segment of the polypeptide must thread through a series of narrow channels and pores, first in the ATPase ring and then in the outer α-subunit ring of the CP (Fig. 1). Importantly, these channels are misaligned and obstructed in the “resting” proteasome.

At the heart of the conformational gymnastics of the proteasome is the hexameric AAA ATPase ring. Each of the six related “Rpt” subunits (RP ATPase subunit) of the ring has an N-terminal coiled-coil (CC) element, followed by an oligosaccharide/oligonucleotide-binding (OB) domain and an AAA domain; the latter comprises a large domain and a smaller α-helical domain, which both contribute to nucleotide binding. The OB domains form a rigid ring linked by flexible linkers to the AAA-domain ring; the AAA domains arrange in an asymmetrical lock-washer arrangement that is dynamic (10). Three conformational states of the yeast proteasome have previously been suggested: s1 (substrate recruitment), s2 (commitment), and s3 (enzymatic processing) (1, 5). These conformational states have been inferred primarily from differences in positioning of the ubiquitin receptors, alignment of the central channels of the OB and AAA rings with the CP channel, and movement of the Rpn11 active site over the OB ring channel.

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So what exactly do these new, higher resolution structures tell us about how the proteasome works? The papers by Schweitzer et al. (1) and Huang et al. (2) describe a single conformation, which appears to be identical to the $S_A$ conformation seen for over 75% of the particles by Chen et al. (3) (Fig. 1); it is roughly equivalent to the $s_1$ state reported for the yeast proteasome. Notably, all three reports include the surprising observation that despite engagement of the RP ATPase ring with the CP outer ring, the gate of the CP $\alpha$-ring remains closed. RP-CP binding was thought to be sufficient for CP gate opening, but at least for the human proteasome, RP binding does not necessarily open the gate. Key contacts between these rings come from the C-terminal tails of specific Rpt subunits. The Rpt2, Rpt3, and Rpt5 subunits end with a conserved HbYX (hydrophobic-Tyr-any residue) sequence that inserts into specific pockets on the $\alpha$-ring surface (11). In the $s_1$ and $S_A$-$S_C$ states, only two of these three tails are stably inserted (the yeast Rpt5 and human Rpt2 do not show tail density in the $\alpha$-ring pockets). By contrast, Chen et al. (3) report that in the $S_0$ state, each ATPase subunit tail except the Rpt4 tail has inserted into CP surface pockets, and the $\alpha$-ring is opened. This fuller tail engagement is made possible through coordinated movements of the ATPase ring on the $\alpha$-ring surface in the $S_C$ and $S_0$ states that involve stepwise axial rotation as well as translational movements and tilting of the ATPase ring into alignment with the $\alpha$-ring.

Changes in the lid-base interface are expected to coordinate the deubiquitylation of substrates by Rpn11 with translocation of the substrate through the rings formed by the OB and AAA domains of the ATPase subcomplex (5, 9) (Fig. 1). Much of this interaction surface centers on the CC-OB domains of the Rpt3–Rpt6 ATPase dimer, which acts as a fulcrum for rotations of the lid relative to the base. Pronounced movements are seen among the $S_A$, $S_B$, $S_C$, and $S_D$ states. For example, a $40^\circ$ rotation of the lid occurs in the switch from the $S_A$ to $S_B$ state, helping to reposition the Rpn11 active site; the $S_B$ state is roughly equivalent to the yeast $s_2$ state.

What is not yet clear from these structures is how ATP binding and hydrolysis are driving the conformational cycle of the proteasome holoenzyme. In the AAA ATPases, ATPase conformations, especially the relative spatial arrangement of the large and small AAA domains, change in response to nucleotide state and site occupancy (12). In the reported proteasome structures, nucleotide could be modeled in all six ATPase subunits, but the resolution was insufficient to designate it as ATP or ADP or some intermediate state [although ADP was unambiguously modeled in Rpt6 in the study by Schweitzer et al. (1)]. Because many particles were averaged to derive the structural models, mixtures of binding states would be hard to distinguish at the resolutions available. No specific substrate has been trapped on the proteasome in any of the new studies. It will be important in future work to determine how substrate bound at different stages of passage through the proteasome affects the relative frequency of the various conformational states and how these changes relate to ATP binding and hydrolysis. Higher resolution structures will be necessary for this determination.

Chen et al. (3) propose a model that relates the four conformational states of the human proteasome to stepwise changes in the functional status of the complex during substrate binding, deubiquitylation,
translocation, and degradation (Fig. 1). In this model, binding of substrate to the S\textsubscript{A} "ground state" is followed by a series of conformation changes (to the S\textsubscript{B} and S\textsubscript{C} states) that remodel the proteasomal subcomplex interfaces and place the Rpn11 subunit in position to deubiquitylate the substrate and realign the channels within the OB and AAA domains. Loops lining the interior of the OB and the AAA rings create narrow openings for substrate passage. The AAA domains display a spiral arrangement of the aromatic-hydrophobic (Ar-Φ) residue-containing loops thought to drive substrate translocation through the ring (12). Two of the ATPase HbYX tails have inserted into the CP α-ring surface in these states, but this insertion is not sufficient for gate opening. Only upon additional translational and rotational movements of the ATPases, possibly triggered by ATP-dependent lid/base and lid/CP rearrangements, can additional Rpt tails insert into the α-ring and promote gate opening.

The new cryo-EM structural findings, particularly in the study by Chen et al. (3), provide a valuable structural framework for further mechanistic analysis of the proteasome. Still higher resolution structures will, of course, be welcome, but other methods, such as quantitative chemical cross-linking (13) and solution biophysical methods, will be needed to validate the new conformational states proposed by Chen et al. (3) and to link substrate unfolding, deubiquitylation, and translocation with the ATPase cycles of the RP. It is safe to say there is still much to be learned about this fascinating proteolytic machine.