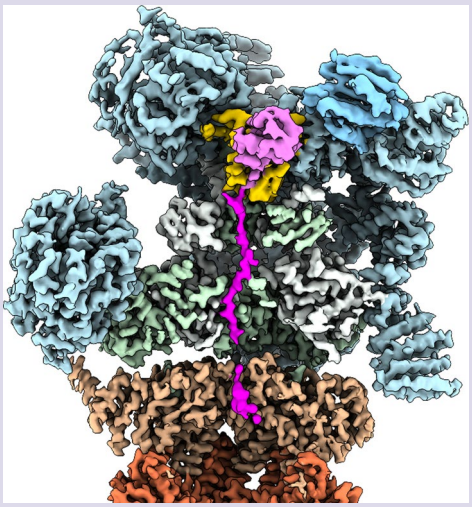
PROTEASOME

Substrate-engaged 26S proteasome

At the center of the eukaryotic cellular proteostasis network, and at the end of many a protein's life, the 26S proteasome catalyzes the degradation of ubiquitin-tagged polypeptides. Two groups have recently used cryo-EM to offer the first high-resolution views of the substrate's interaction with the proteasome and insights into the inner workings of this macromolecular machine: de la Peña et al. (*Science* https://doi.org/10.1126/science.aav0725) report structures of the substrate-engaged 26S proteasome from yeast (pictured, with substrate in magenta), whereas Dong et al. (*Nature* https://doi.org/10.1038/s41586-018-0736-4) present the structure of a comparable human complex.



Credit: Gabriel C. Lander, The Scripps Research Institute, San Diego, CA, USA

The 26S proteasome is composed of the double-barrel-shaped proteolytic 20S core particle (CP, shown in wheat and orange) and the 19S regulatory particle (RP, shown in blue, white and teal), which binds at one or both ends of the CP and recruits, deubiquitinates and unfolds the substrates, then threads them into the CP. The RP can be further divided into a base and a lid. The base functions as the molecular motor of the proteasome and includes a ring of six subunits (Rpt1–Rpt6, shown in white and teal) from the ATPases associated with diverse cellular activities (AAA+) family. Several AAA+ members have been visualized forming a spiral staircase, and nucleotide-bound subunits in the ring are thought to contact the substrate through loops extending onto the central pore, while the nucleotide-free 'seam' subunit remains disengaged from the substrate.

To trap the substrate inside the proteasome, de la Peña et al. chemically inhibited the Rpn11 deubiquitinase (shown in yellow, bound to ubiquitin moiety shown in pink) within the RP lid subcomplex. In contrast, Dong et al. incubated human proteasomes with a polyubiquitinated model substrate and ATP, and then switched to slowly hydrolyzable ATP-γS before preparing samples for imaging.

In both reports, the authors clearly observed a spiral-staircase arrangement of individual Rpt subunits, as well as their interactions with one another, with the substrate (through Rpts' pore loops) and with the outer ring of the CP (through Rpts' C-terminal tails). The outer ring forms a gate to the CP cavity, captured in distinct (open, partially open or closed) states, thus providing new insights into the mechanisms of gating.

The reported distinct conformations of the AAA+ motor, the various nucleotide states of the Rpt subunits around the hexameric ring and their coupling to substrate binding reveal how RP drives substrate translocation. de la Peña et al. have found that the disengaged seam subunit and the subunit at the bottom of the staircase are ADP bound, and the remaining four Rpts are ATP bound. They propose that nucleotide exchange in the seam subunit primes it for substrate binding at the top of the staircase. Simultaneously, the second-to-bottom subunit hydrolyzes ATP, and subsequent phosphate release triggers major conformational changes resulting in substrate translocation.

Dong et al. have also observed that most of the substrate-engaged subunits are ATP bound, whereas the subunit at the bottom of the staircase is ADP bound. On the basis of the captured proteasome conformations, they propose that ATP hydrolysis in the proteasome occurs differently at different stages of substrate processing. First, coordinated ATP hydrolysis in a pair of diametrically opposed AAA+ subunits promotes substrate recognition and deubiquitination. Coordinated ATP hydrolysis in two adjacent subunits initiates substrate translocation and coordinates CP gating. In later steps, sequential hydrolysis of one nucleotide at a time and the coordinated ADP release from the adjacent subunit fuel substrate translocation in a processive manner.

Both groups propose that processive substrate translocation occurs through the rotation of the substrate-bound ATPases, a movement compared by de la Peña et al. to the conveyor-belt mechanisms suggested for other AAA+ motors. Together, the two papers advance understanding of the mechanisms of substrate recognition, engagement and translocation by the eukaryotic proteasome. ❐

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