*Nature Reviews Molecular Cell Biology* | AOP, published online 10 June 2009; doi:10.1038/nrm2719

PROTEIN DEGRADATION

# Assembly from the base

> …RP assembly in yeast and mammals requires multiple chaperones…

The 26S proteasome comprises the 20S proteolytic core particle (CP) and the 19S regulatory particle (RP), which consists of a lid subcomplex and a base subcomplex. Whereas the mechanisms underlying CP assembly are well established, little is known about how the RP forms. Several groups now report that RP assembly in yeast and mammals requires multiple chaperones that facilitate the formation of specific subassemblies, which subsequently assemble into the proteasome base and the RP.

Mass spectrometric analysis of immunopurified proteasomal complexes from budding yeast, carried out by Funakoshi, Saeki, Roelofs and colleagues, reveals four proteasome-interacting proteins (PIPs) — Nas6 (gankyrin or p28 in mammals), Rpn14 (PAAF1 in mammals), Hsm3 (S5B in mammals) and, in the first two studies, Nas2 (p27 in mammals). These PIPs bind specifically to free RP or RP base subcomplexes but are absent from the mature 26S proteasome. Each of these proteins binds to distinct subunits of the base, which suggests that they could be base-specific chaperones.

Phenotypic analysis of different mutant strains indicates that PIPs have overlapping roles in 26S proteasome function, and the three groups show that double, triple and quadruple mutants are defective in assembling the 26S proteasome *in vivo*. Excess free lid, CP and base subcomplexes were detected in specific multiply mutated strains, but the RP and the full 26S proteasome were depleted. This indicates defective base assembly and therefore confirms that the PIPs are base-specific chaperones.

The groups further show that each chaperone forms specific subassemblies, as detected by native gels and mass spectrometry analysis, and that these subassemblies seem to associate in an ordered manner to form the base subcomplex. Similar findings are described by Kaneko *et al.*, who studied the assembly of the mammalian base subcomplex. Together, they demonstrate the remarkable evolutionary conservation in the assembly pathway.

So, how is assembly of the base subcomplex connected to the assembly of the RP and the 26S proteasome? Base-specific chaperones bind near the carboxy-terminal domains of specific base subunits. These subunits, Rpt1–6, form an ATPase ring on the CP with their C termini inserted into binding pockets in the CP. Park *et al.* show that mutants that lack the C-terminal residue of the Rpt subunits accumulate free CP and free lid, as seen with the chaperone mutants. These and other findings show that base assembly depends on the interaction with the CP. The mechanistic link between the chaperones and the C termini of the Rpt subunits is provided by Roelofs *et al.*, who show that base-specific chaperones can restrict the accessibility of the Rpt C termini to the CP, and that competition between the CP and chaperones might explain the release of chaperones as the 26S proteasome matures.

*Arianne Heinrichs*

**ORIGINAL RESEARCH PAPERS** Funakoshi, M. *et al.* Multiple assembly chaperones govern biogenesis of the proteasome regulatory particle base. *Cell* **137**, 887–899 (2009) | Saeki, Y. *et al.* Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle. *Cell* **137**, 900–913 (2009) | Roelofs, J. *et al.* Chaperone-mediated pathway of proteasome regulatory particle assembly. *Nature* **459**, 861–865 (2009) | Kaneko, T. *et al.* Assembly pathway of the mammalian proteasome base subcomplex is mediated by multiple specific chaperones. *Cell* **137**, 914–925 (2009) | Park, S. *et al.* Hexameric assembly of the proteasomal ATPases is templated through their C termini. *Nature* **459**, 866–870 (2009)
**FURTHER READING** Murata, S. *et al.* Molecular mechanisms of proteasome assembly. *Nature Rev. Mol. Cell Biol.* **10**, 104–115 (2009)