RPN10, and Subunit Interaction Maps of 26S Proteasome and Cop9 Complexes

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By degrading critical regulatory factors, the ubiquitin-mediated proteolysis plays central roles in many cellular processes. The 26S proteasome, a multisubunit complex, is the primary protease responsible for degrading ubiquitin-tagged proteins. The complex is composed of two relatively stable subparticles: the 20S core particle (CP), a hollow cylindrical structure which contains the proteolytic active sites in its lumen, and the 19S regulatory particle (RP) which binds to either end of the CP and provides the ATP-dependence and specificity for ubiquitinated proteins. The RP can further dissociate into stable lid and base subcomplexes. Besides subunit composition and low-resolution EM images, we know very little of structural bases for the RP. To help understand how the RP function, structural resolution of this complex is essential. We have performed detail structural/functional analyses for one of the RP subunits, Rpn10. Whereas a ubiquitin interacting motif (UIM) containing a stretch of hydrophobic residues was located to the C-terminal half, the *in vivo* function of this motif is not known. In contrast, evidence indicates the N-terminal vWA domain is critical for examined *in vivo* functions of Rpn10. A single residue in the vWA domain of Rpn10 is essential for amino acid-analog resistance, for degrading a uiquitin-fusion degradation substrate, and for stabilizing lid-base association. To help define the molecular organization of the RP, we tested all possible paired interactions among subunits from *Saccharomyces cerevisiae* by yeast 2-hybrid analysis. Within the base, a Rpt4/5/3/6 interaction cluster was evident. Within the lid, a structural cluster formed around Rpn5/11/9/8. Interactions were detected among synonymous subunits (Csn4/5/7/6) from the evolutionarily related COP9-signalosome (CSN) from Arabidopsis, implying a similar quaternary arrangement. No paired interactions were detected between lid, base or CP subcomplexes, suggesting that stable contacts between them require prior assembly. Mutational analysis defined the ATPase, coiled-coil, PCI, and MPN domains as important for RP assembly. Comprehensive subunit interaction maps for the 26S proteasome and CSN support the ancestral relationship of these two complexes.